

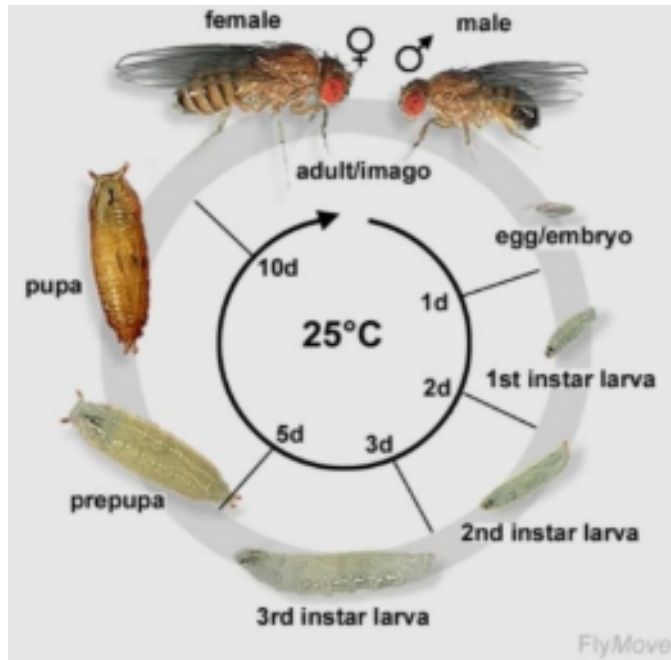
The importance of DNA



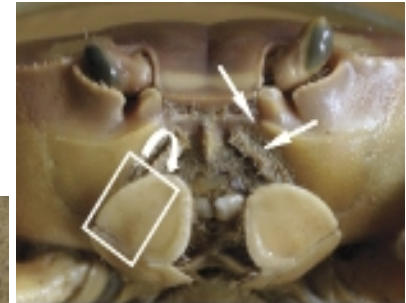
Genetics

V. Courtier-
Orgogozo

My research: *Drosophila* glue



crab



soil



D. grimshawi

rotten strawberry



D. suzukii

grape



D. melanogaster

potato

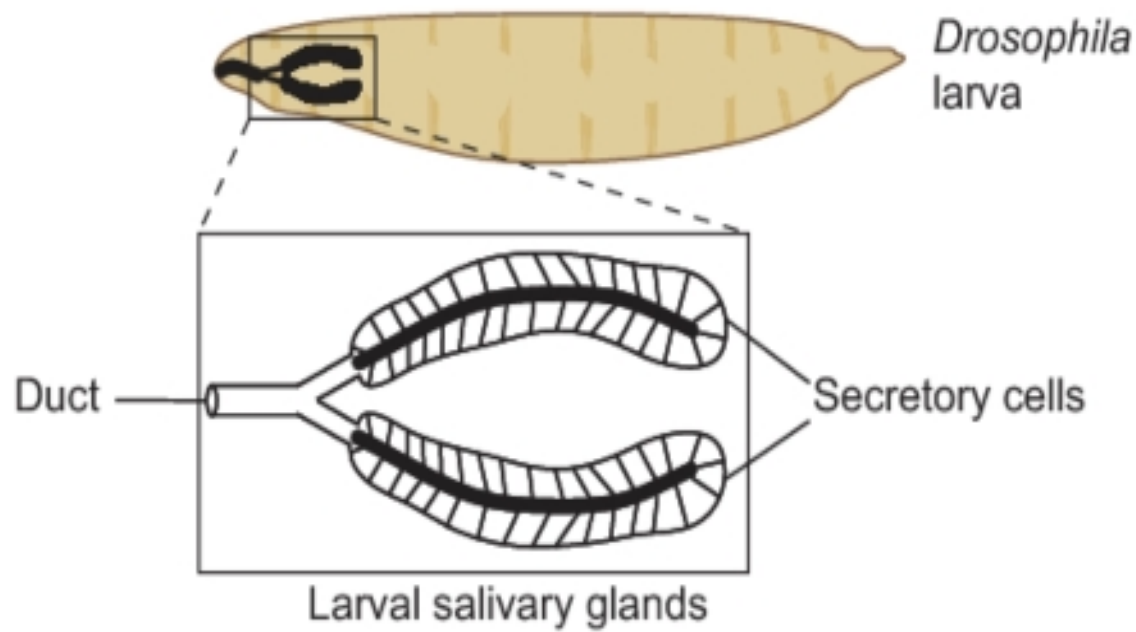


D. repleta



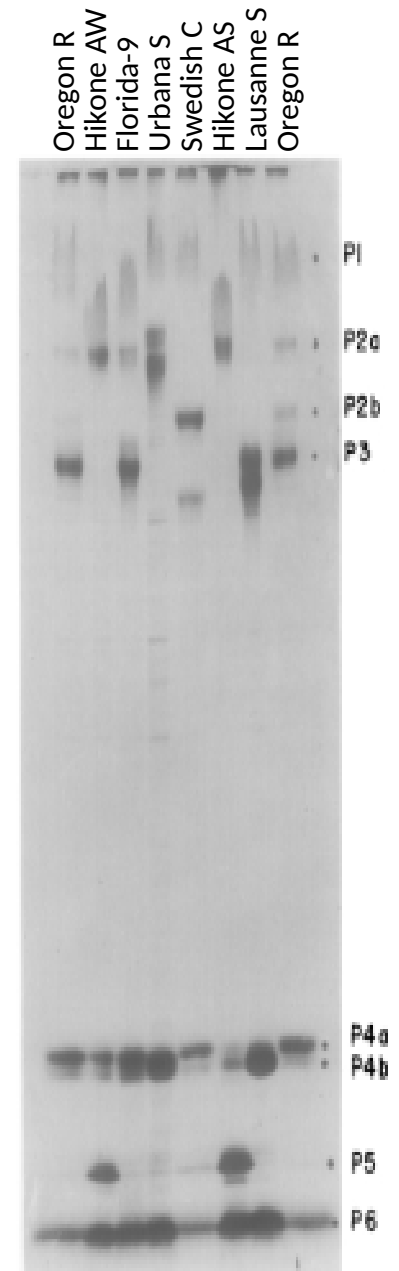
D. carcinophila

Glue proteins vary greatly between *D. melanogaster* strains



Tran & Hagen 2017

Extraction of salivary
content
acrylamide gel
protein staining



Beckendorf & Kafatos 1976

Bioadhesives

Natural polymer that can act as an adhesive: binds two items together and resists their separation



Mussel glue

water resistant

25 proteins

3,4-dihydroxyphenylalanine (DOPA)

to Understand Adaptation

A model &

to Develop New Bioadhesives

Genes mediating
interactions
with the environment
Immunity
Stress response
Reproduction

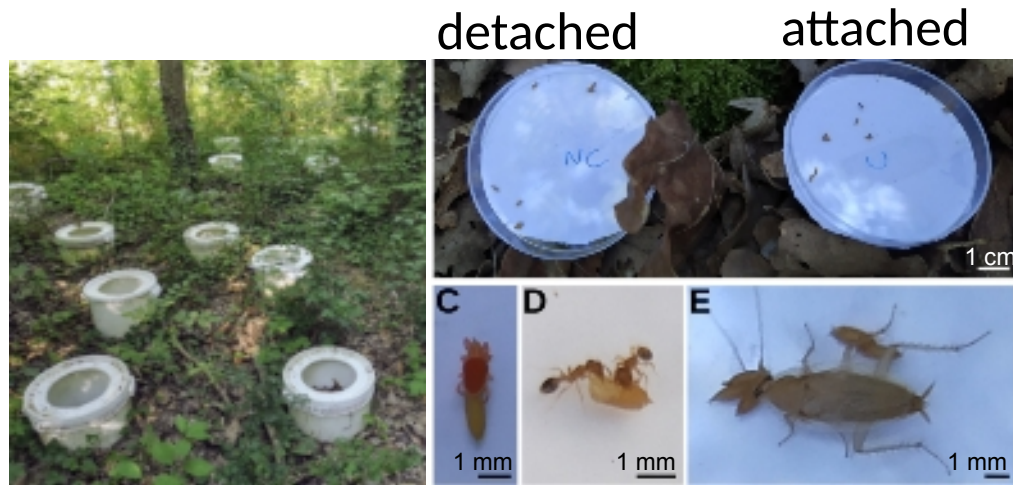
Rapidly evolving

Most studied bioadhesive
Mussel glue (water)



Function of the glue?
How adhesive is the glue?
What makes it adhesive?

In forest: detached pupae are gone more rapidly



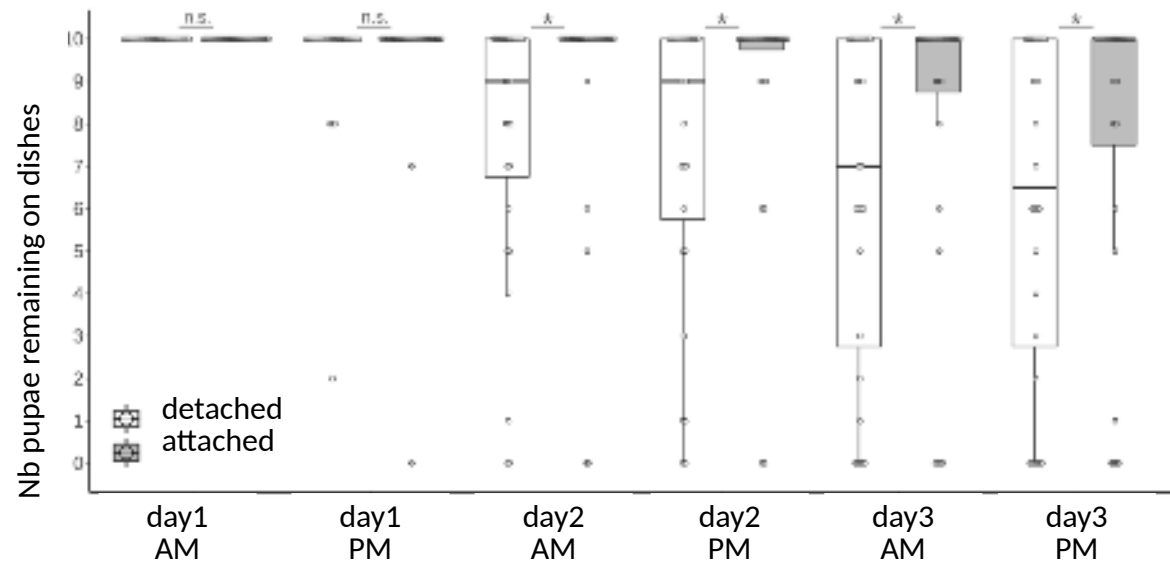
In Bois de Vincennes (Paris)

In each bucket (n=28):

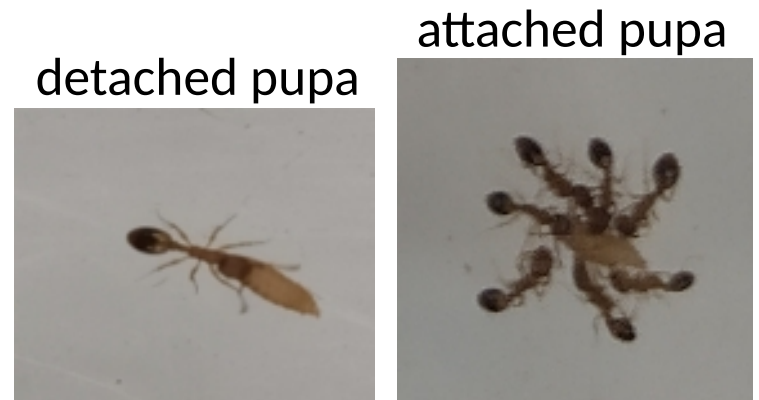
one Petri dish w/ 10 detached pupae

one Petri dish w/ 10 attached pupae

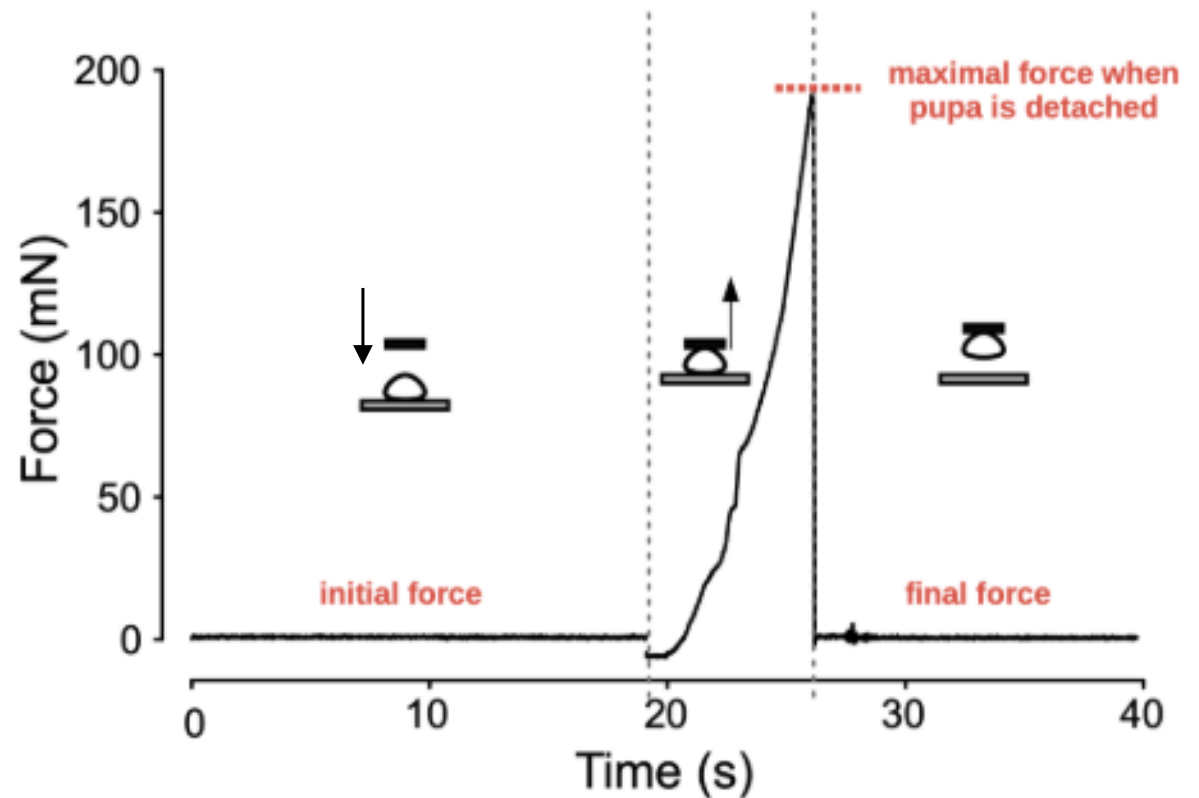
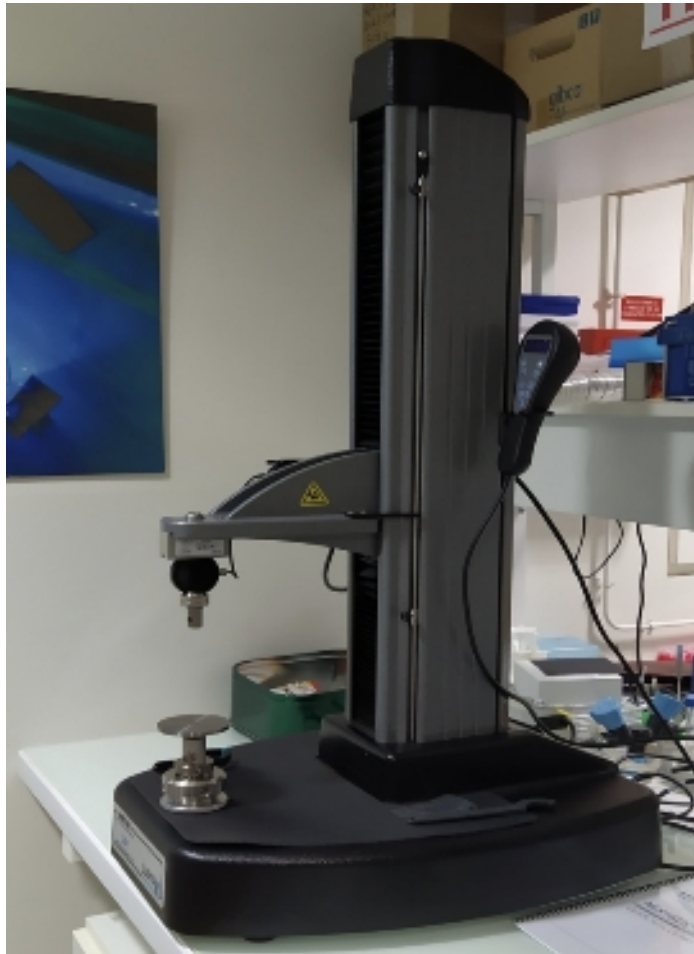
Checked twice a day



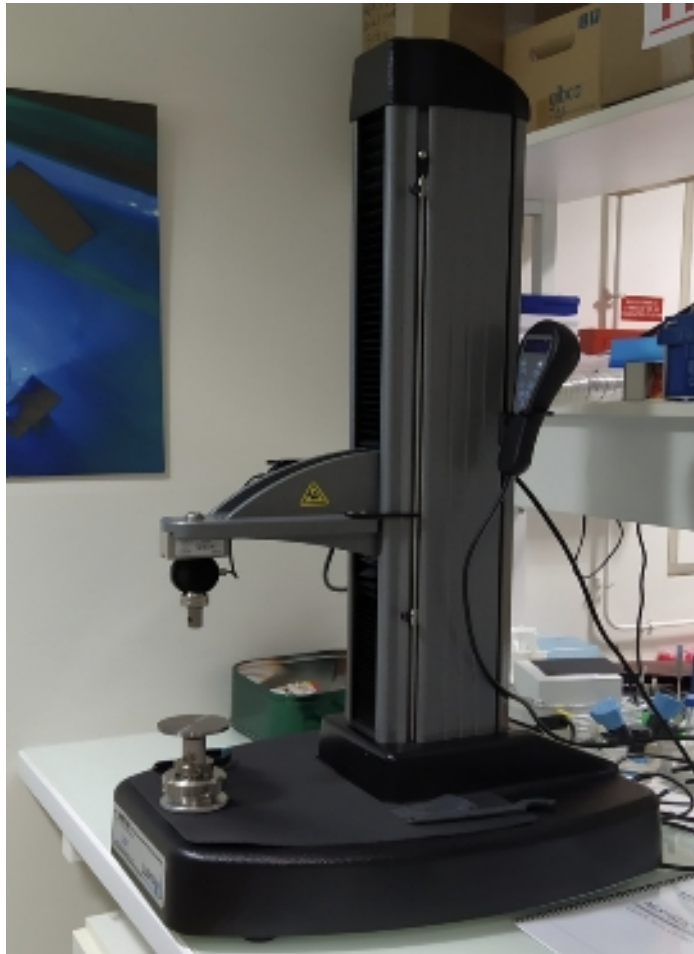
In the lab:



A new method to measure *Drosophila* glue adhesion



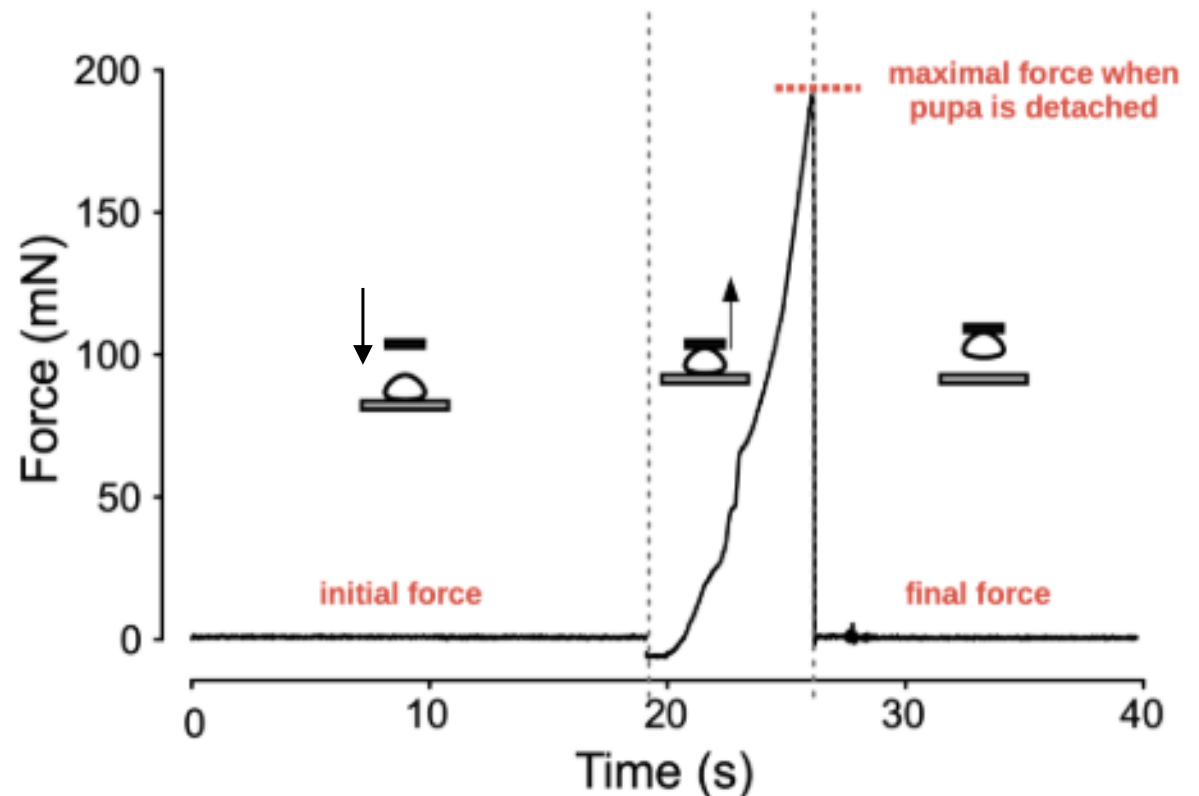
D. melanogaster glue sticks as strongly as our most adhesive tapes



100-300kPa

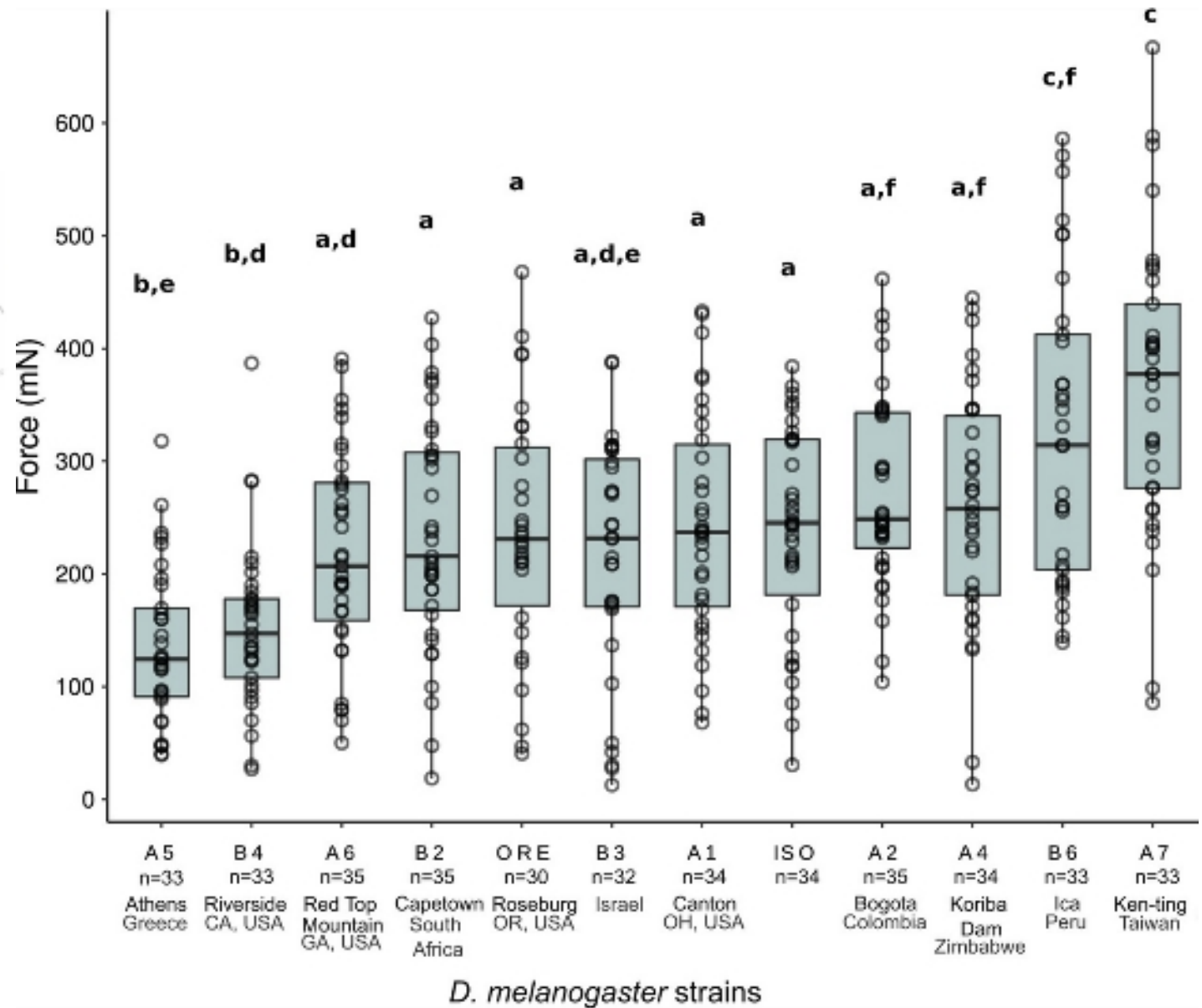


10MPa

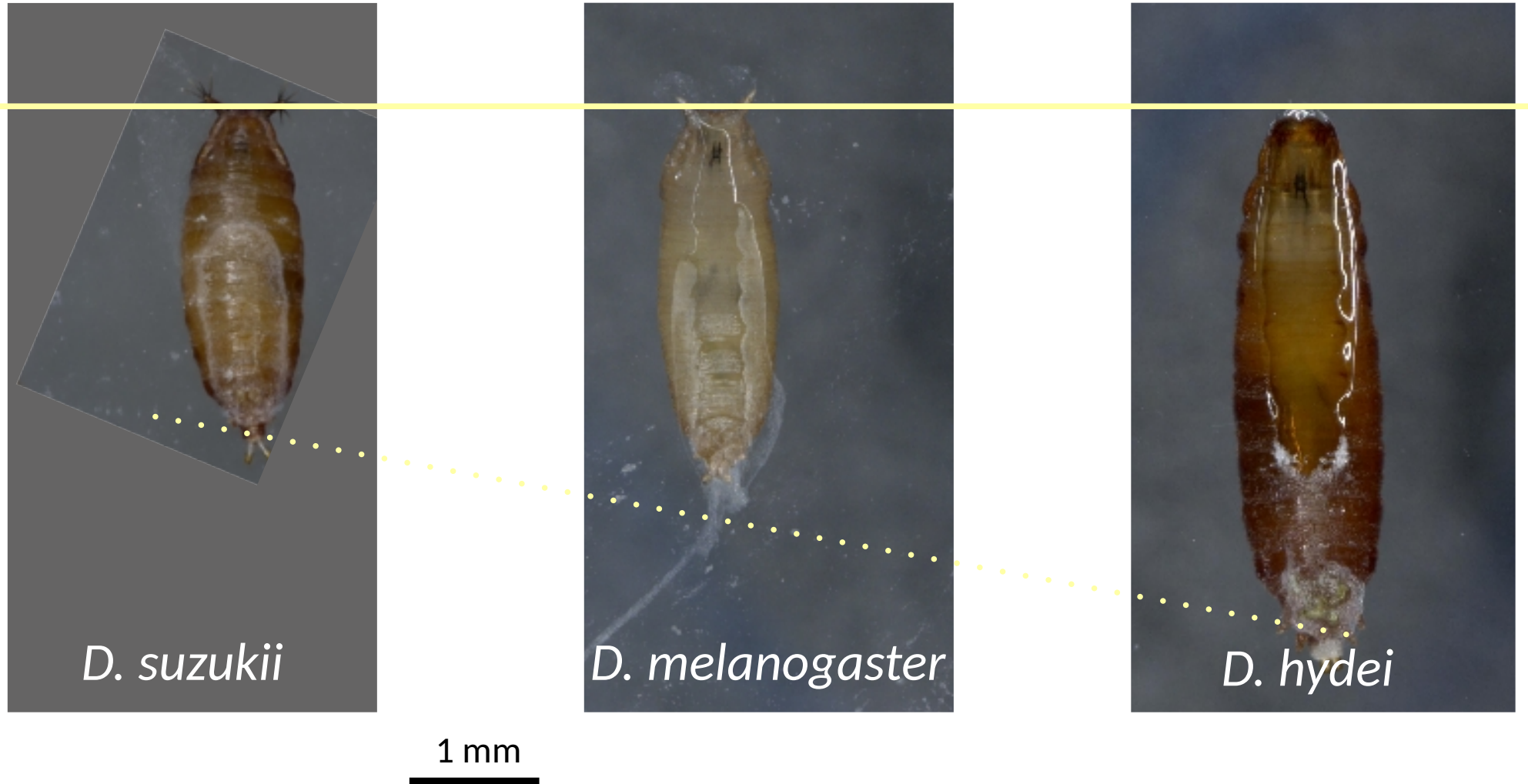


Adhesion force varies within species

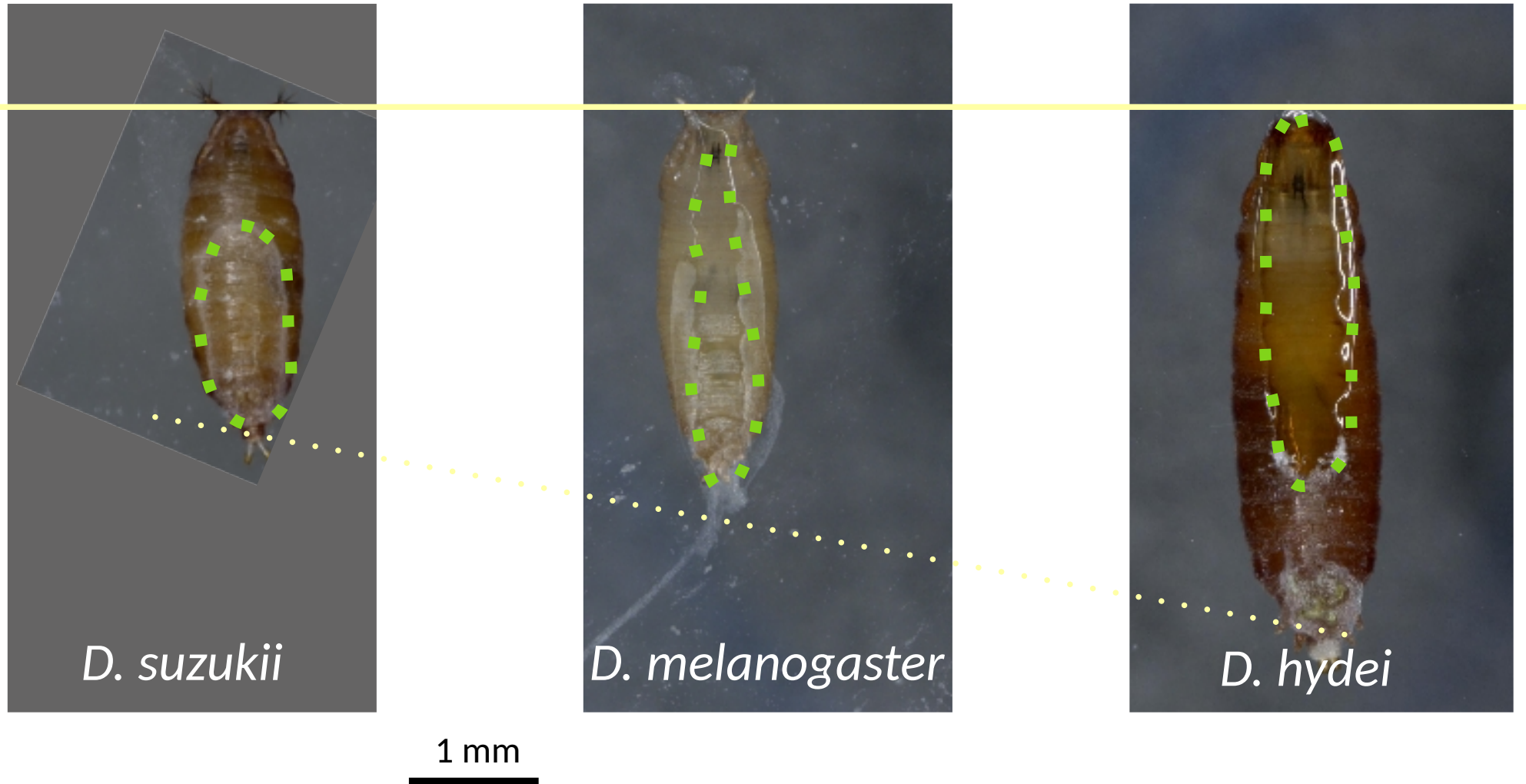
D. melanogaster lines
DSPR



Pupa size and glue area vary between species

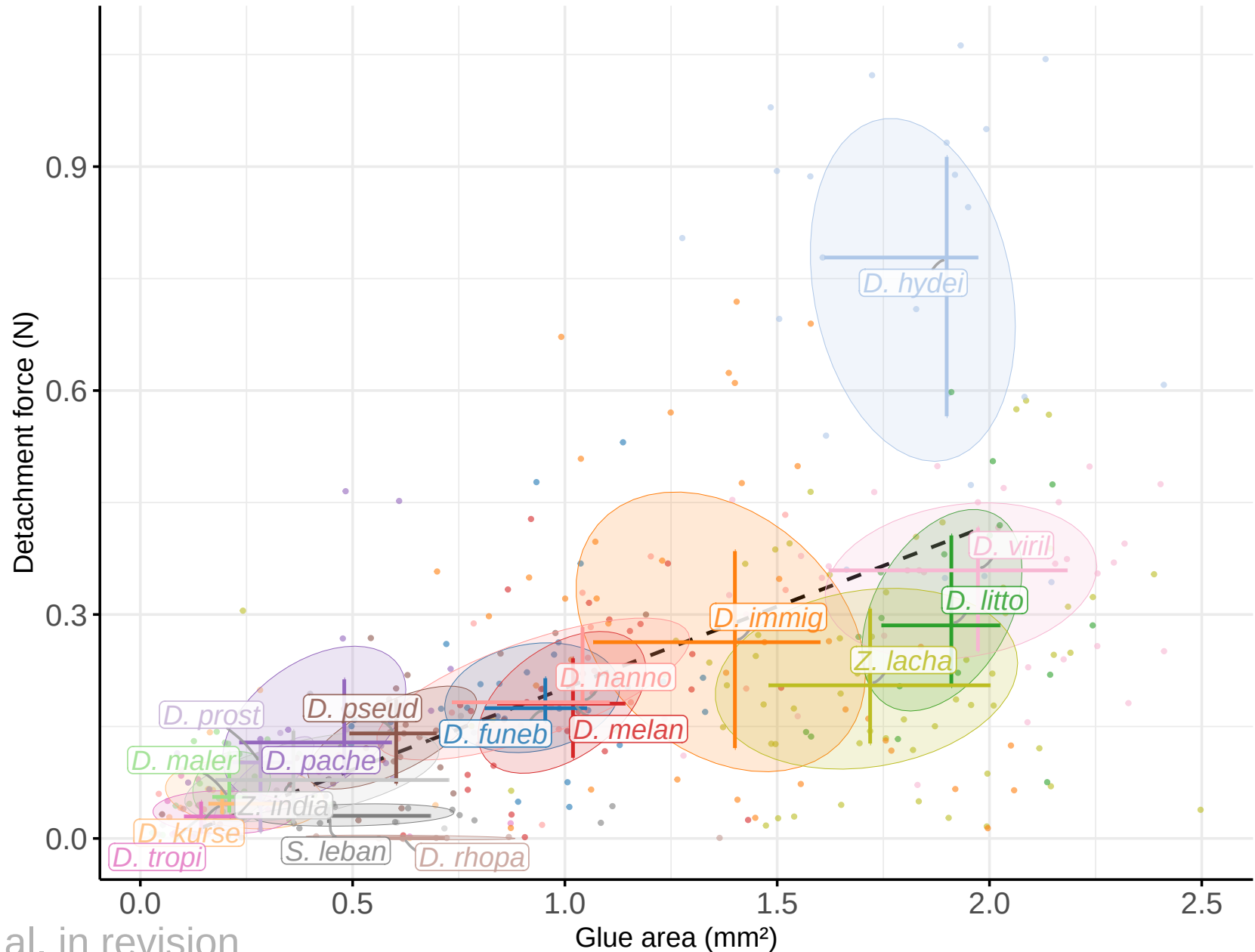


Pupa size and glue area vary between species

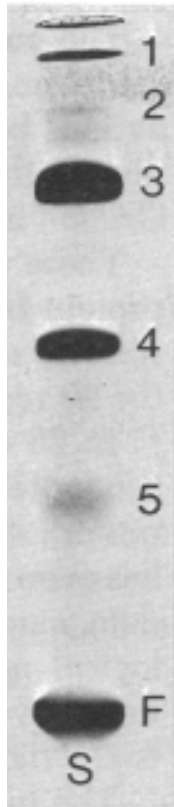


D. hydei has the highest adhesion strength

Adhesion strength = detachment force / glue area



***D. melanogaster* glue is composed of 8 proteins**



Sgs1, Sgs3, Sgs4, Eig71Ee (1286, 307, 287, 445 aa)

Long, repeats rich in Ser, Thr, Pro

Disordered

O-glycosylated

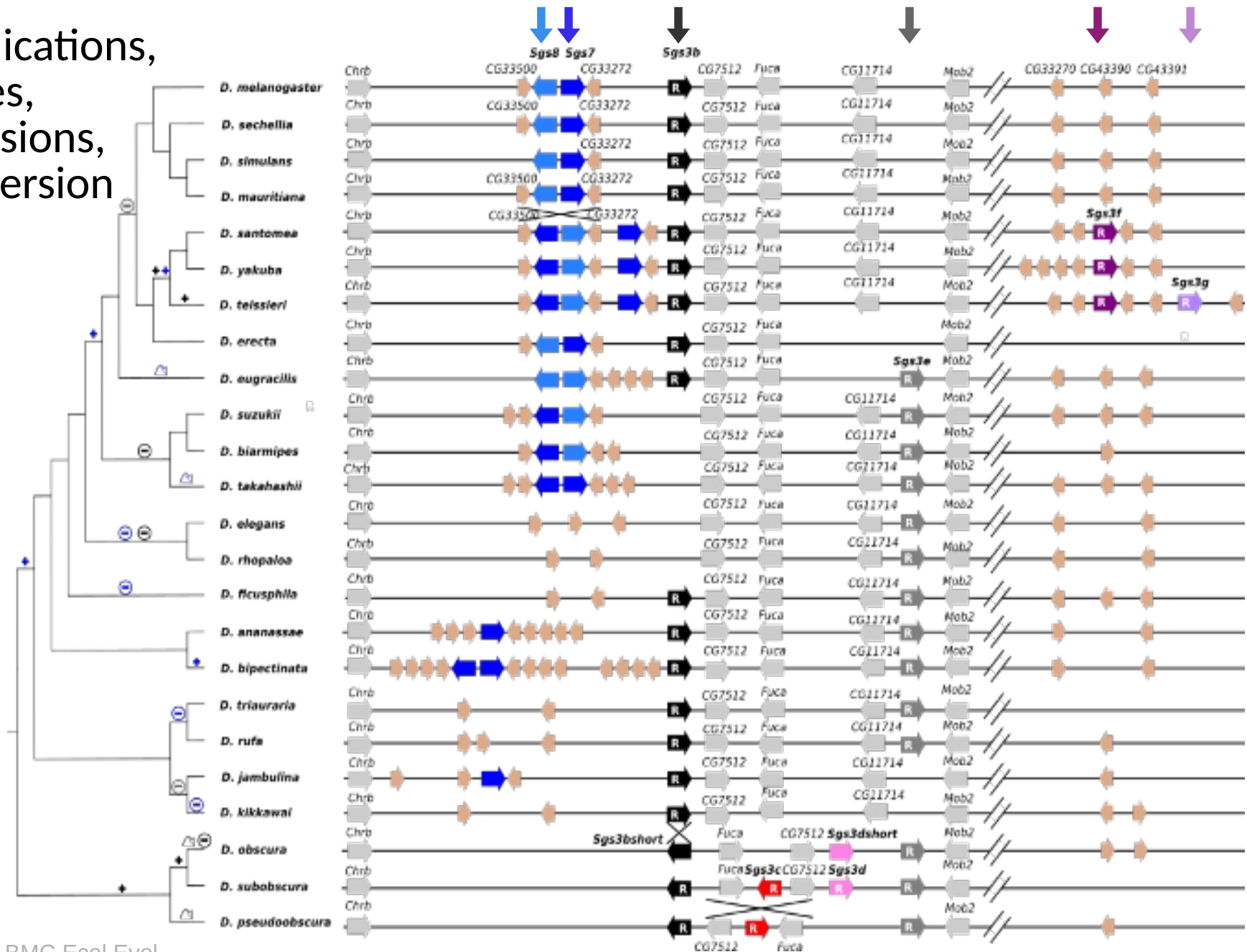
Sgs5, Sgs5bis, Sgs7, Sgs8 (163, 142, 74, 75 aa)

Short, rich in Cys

Korge 1975

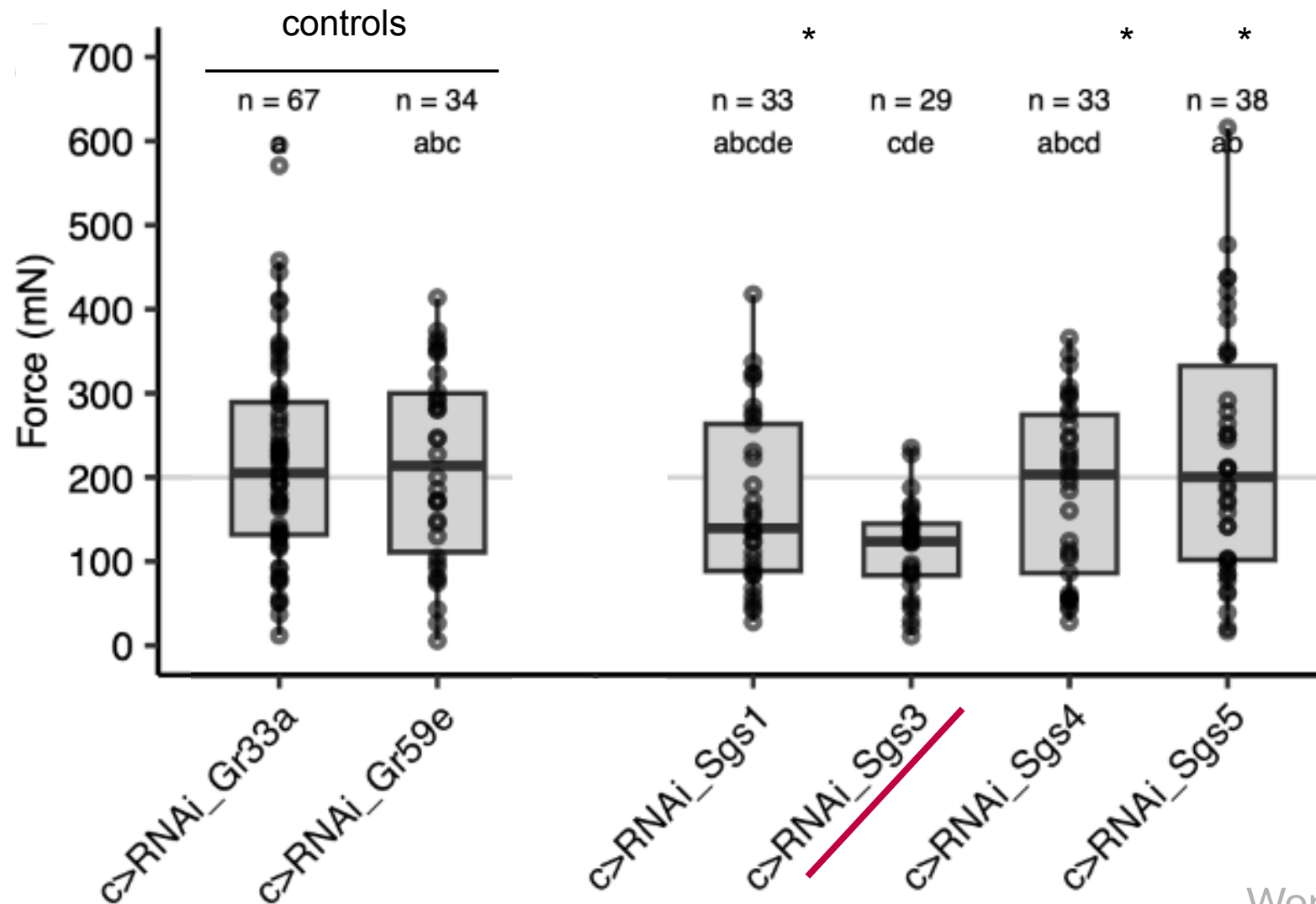
Rapid evolution of glue genes between species

Gene duplications,
gene losses,
local inversions,
gene conversion



Inhibition of 3 glue genes (out of 4 tested) seems to reduce adhesion

Salivary gland-specific *GAL4* x *UAS-RNAi*



Conclusion

Drosophila glue as a model to understand adaptation

- protects from ant predation
- intra- and inter-species variation

to develop new bioadhesives

- as adhesive as most adhesive tapes
- *D. hydei* is the most adhesive
- Sgs3, Sgs7, Sgs8 are good candidates

Perspectives

Genetics
Genomics

Characterize the function of each glue gene (CRISPR)
Identify other potential glue genes (RNAseq)

Physics

Improve the adhesion assay, Test natural substrates

Physiology

Test antimicrobial properties of the glue

Biochemistry

Develop new bioadhesives

The importance of DNA



Genetics

V. Courtier-
Orgogozo

Why is DNA an essential molecule in biology?



***How can a single-letter flip in DNA
lead to dramatic changes?***



Should we be worried about GMOs?



Can genetics help us improve the future?



**Why is DNA
an important molecule
in biology?**

Newsweek, May 23, 2005

*Slide from
S. Gilbert*



SAME DNA. SMALLER CHROMOSOMES.

THE ALL-NEW MIDSIZE H3. LIVING UP TO THE OFF-ROAD REPUTATION HUMMER MADE FAMOUS.
COMING SOON. STARTING AT \$29,500. VEHICLE SHOWN \$30,195.*

HUMMER
LIKE NOTHING ELSE.™

*MSRP. TAX, TITLE, LICENSE, DEALER FEES AND OPTIONAL EQUIPMENT ARE EXTRA. 1-800-REAL-4WD
© GENERAL MOTORS CORPORATION 2005

The importance of DNA in biology

Major basis of heritable variation (genotype-phenotype)

Transmitted (can help reconstruct history)

Present in all living entities (DNA/RNA)

Stable molecule (ancient DNA – oldest = 2 million years, forensic)

String of letters, can be easily analyzed with computers (compared to anatomical traits for taxonomy)

Disclaimer:

DNA is not the cause of everything

Monozygotic twins are not identical

Cardiovascular disease associates better with lifestyle than with DNA sequence (Mozaffarian 2008)

Lung cancer associated with smoking habits

Drug metabolism is mostly due to the microbiome

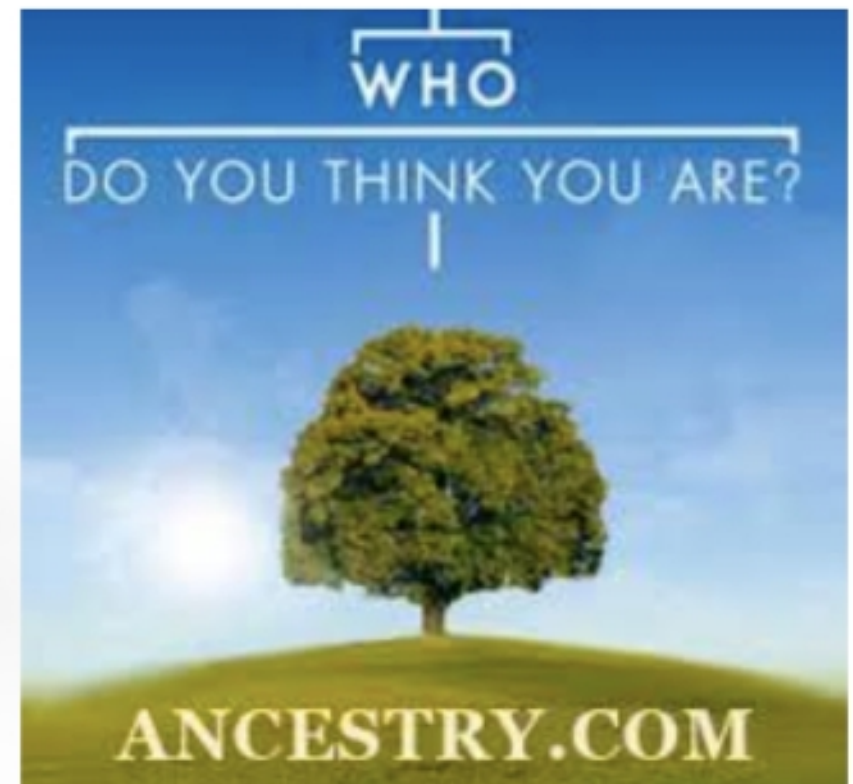
Several genes associated with autism, depression, etc. were “lost” in larger studies

Distilbene: anti-miscarriage drug, increases cancer risks in daughters and malformations in grand-daughters

GENETIC INDIVIDUALITY:

*Slide from
S. Gilbert*

Each of us is a genetically unique individual, and the genes determine who we are.



“...revealing what it is that makes you, you.”

-American television ad for ancestry.com 2015

Manipulating DNA

**What can we do with
DNA ?**

What can we do with DNA ?

Extract, purify

Store

Make more

Amplify

Clone

Synthesize

Examine

Quantify

Examine length

Stain, probe

Sequence

Examine 3D structure

Measure physical properties of DNA molecules

Modify

Cut

Ligate

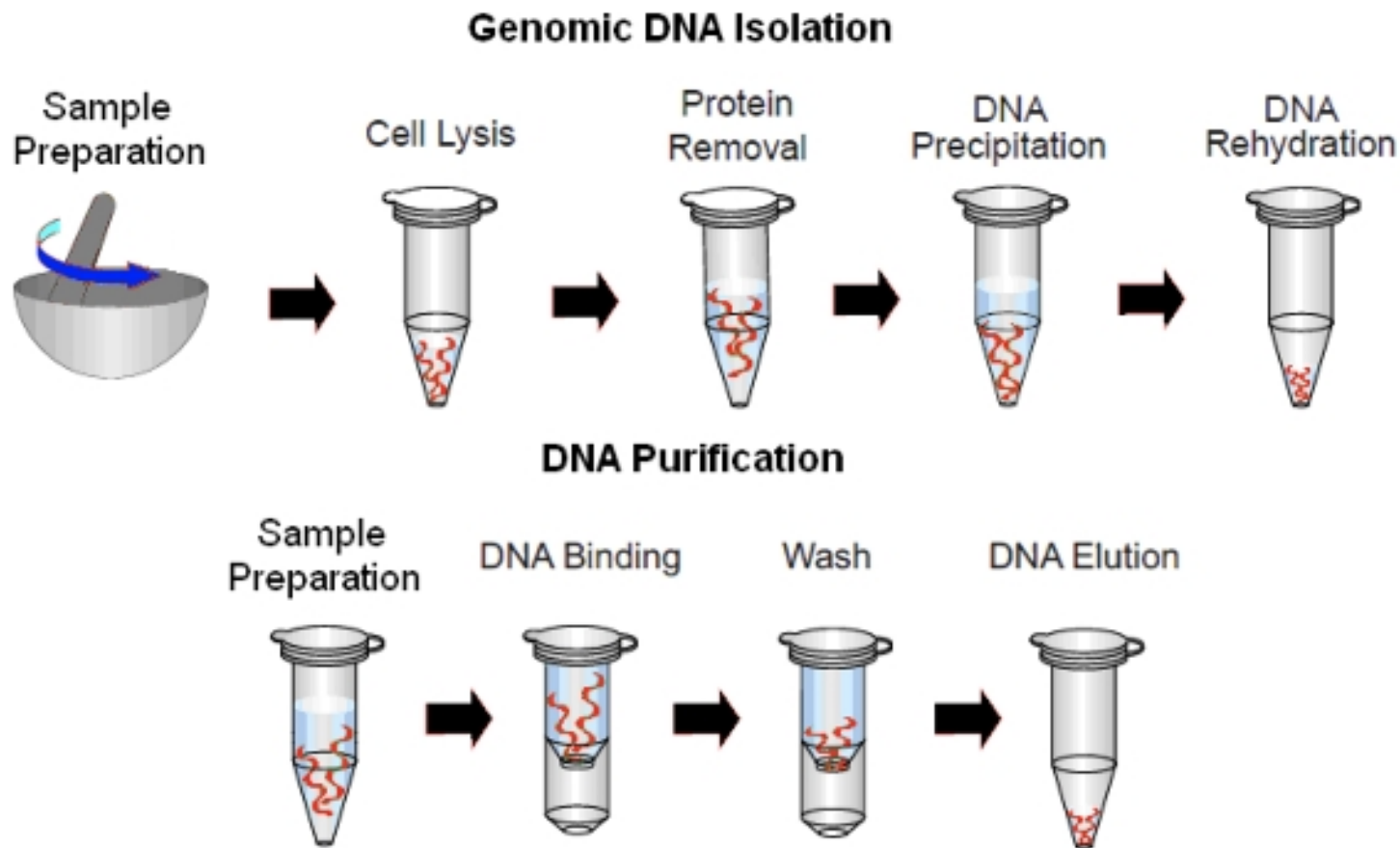
Recombine fragments

Introduce foreign DNA

Mutate

Extract DNA

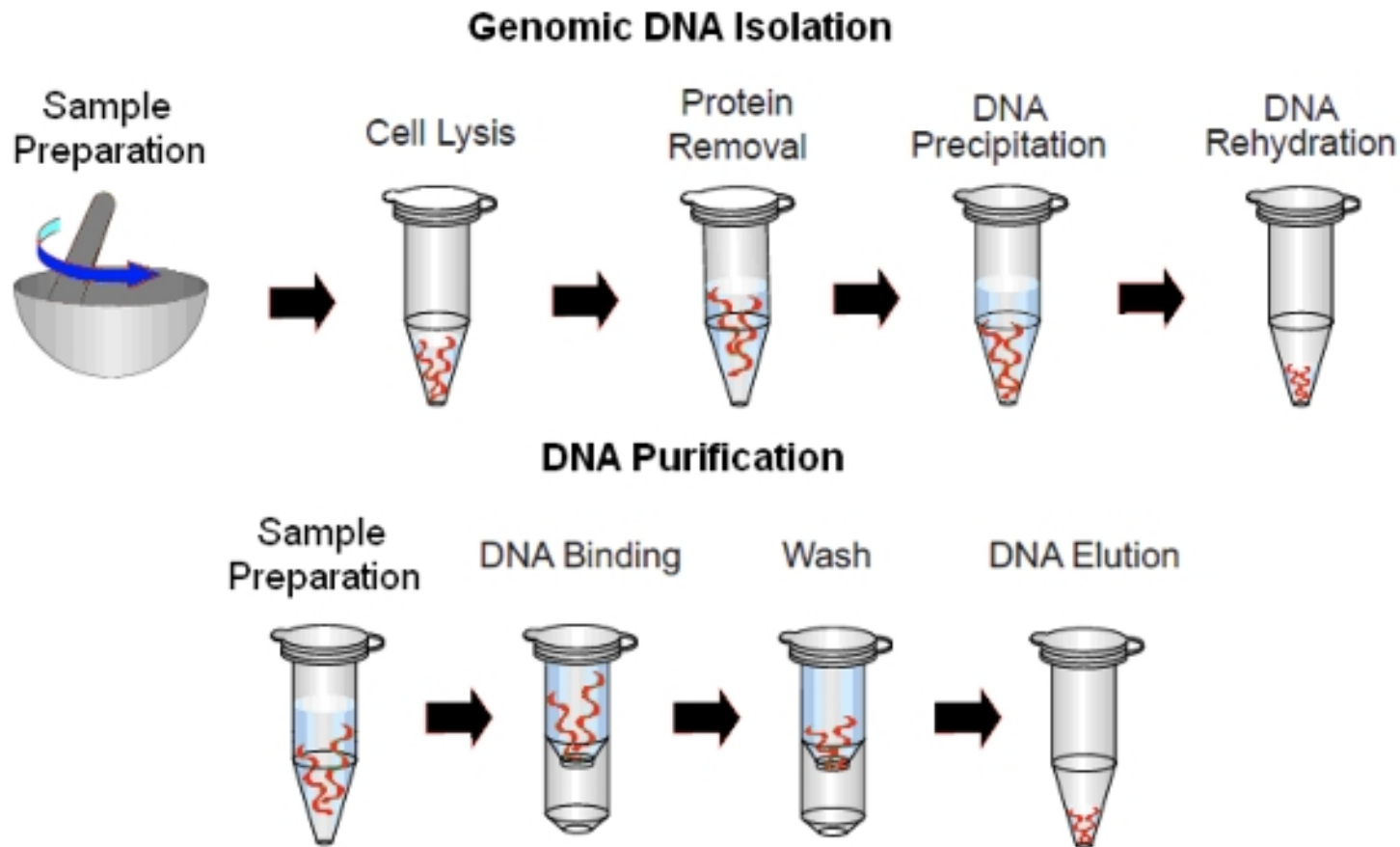
Break cells, remove lipids and proteins,
precipitate DNA, remove liquid, resuspend in aqueous solution



Be aware of contaminants!

Extract DNA

Break cells, remove lipids and proteins,
precipitate DNA, remove liquid, resuspend in aqueous solution



Be aware of contaminants!

(DNA from mitochondria, viruses, bacteria, researcher, symbionts...)

Amplify DNA



Mix:

Genomic DNA

Probes (oligonucleotides)

Nucleotides

Taq polymerase

Ions (MgCl_2)

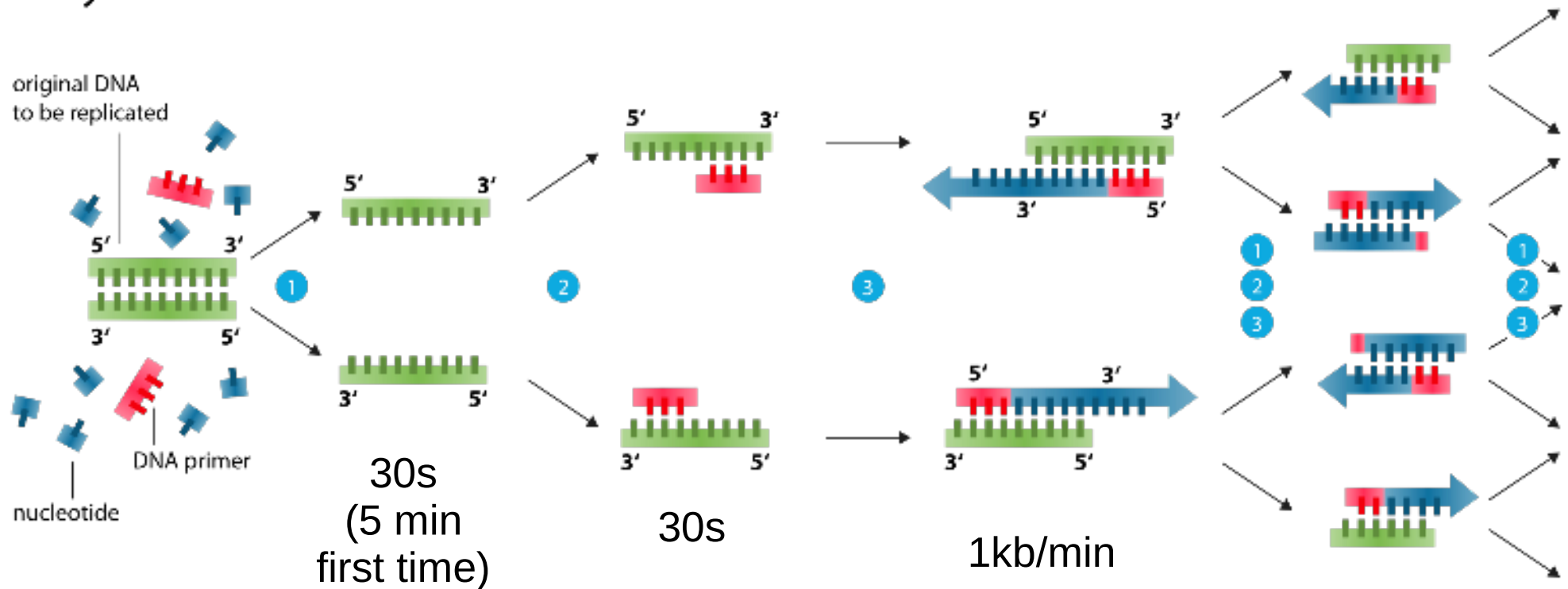
Cycles of Denaturation, Annealing,
Elongation

PCR: Polymerase Chain Reaction

Amplifies DNA fragments of between 0.1 and 10 kb (up to 40 kb)

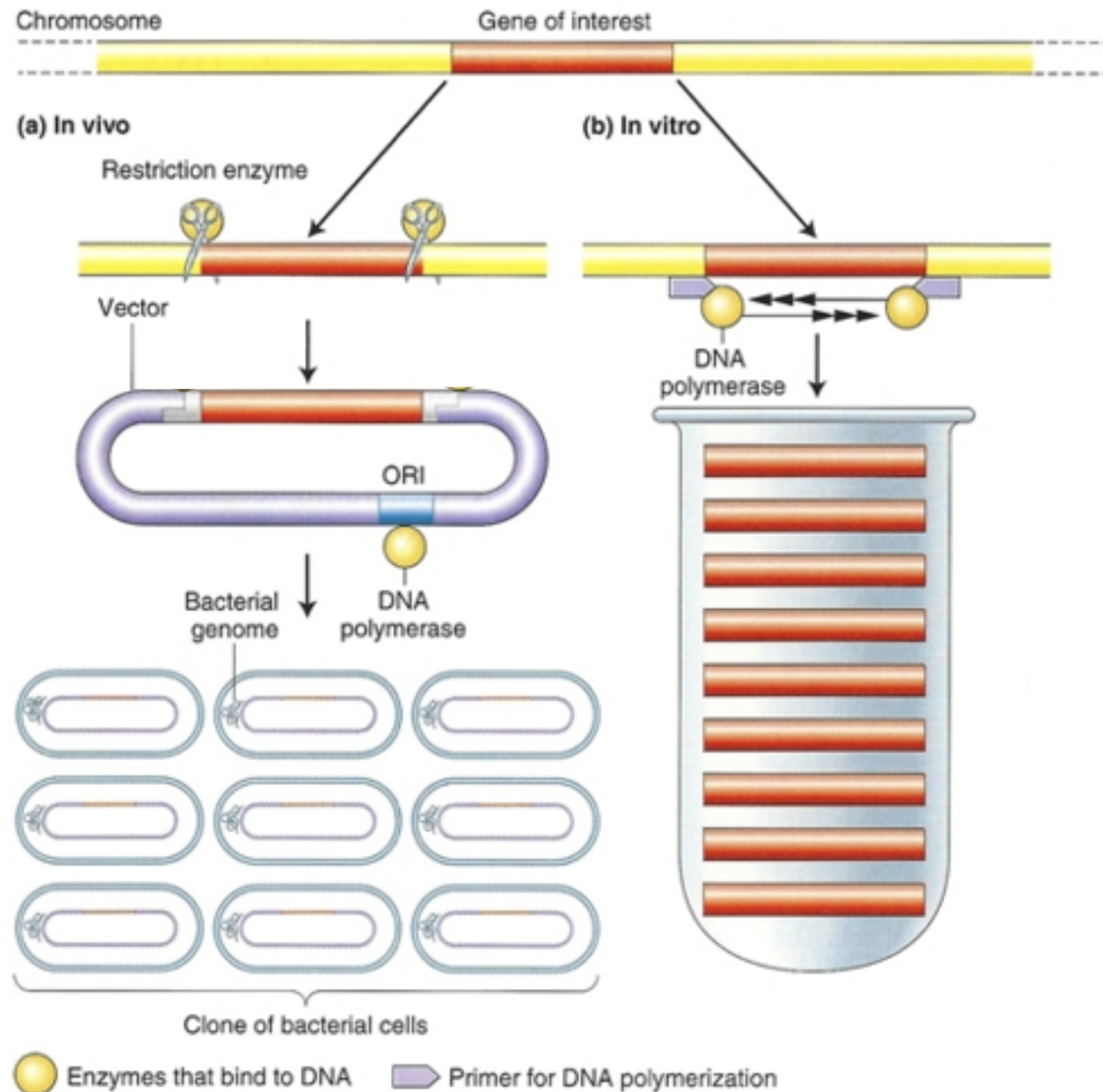
Amplify DNA

Polymerase chain reaction - PCR



- 1 **Denaturation** at 94-96°C
- 2 **Annealing** at ~68°C
- 3 **Elongation** at ca. 72 °C

Cloning vs. PCR



Amplify DNA

DNA fragments

5 kb-15 kb: plasmids in bacteria

~10 kb: lambda phage-based vectors

Up to 40 kb: fosmids in bacteria

~100-300 kb: bacterial artificial chromosomes (BAC)

Commande d'ADN sur internet



	<u>Fast</u>	Standard	<u>Economy</u>	<u>GenBrick</u>
Length	≤ 5 kb	≤ 8 kb	≤ 8 kb	> 8 kb
Turnaround time (starting from)*	5 business days (bd)	8 business days (bd)	10 business days (bd)	23 business days (bd)
Complex genes	✗	✓	✗	✓

Overview

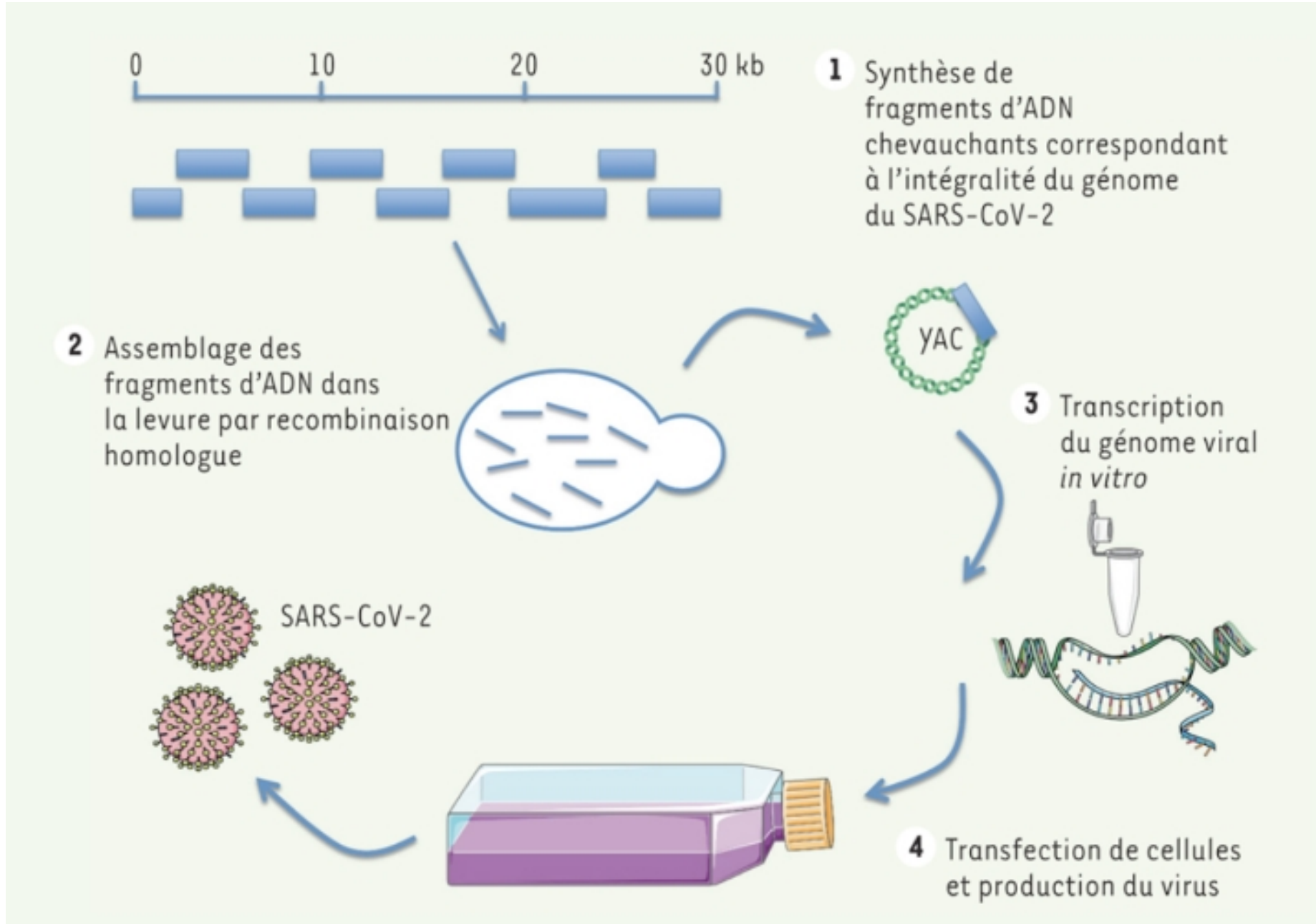
Custom industry-leading genes, 100% sequence accuracy guaranteed

① The standard delivery includes:

- 4 µg of lyophilized plasmid containing your gene insert (1 µg for low-copy plasmid) *
- Sequence chromatograms or NGS read depth plot covering your gene (electronic)
- Construct map for the plasmid (electronic)
- Quality assurance certificate

[https://www.genscript.com/gene_synthesis.html?
src=home](https://www.genscript.com/gene_synthesis.html?src=home)

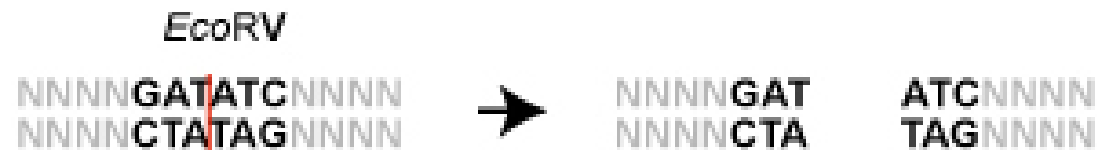
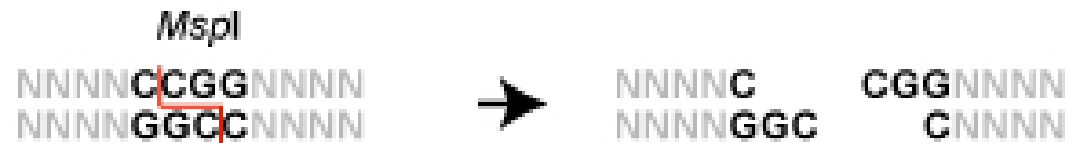
Synthèse de SARS-CoV-2 en un mois



Cut DNA with restriction enzymes

Sites de restriction

Résultats après coupure

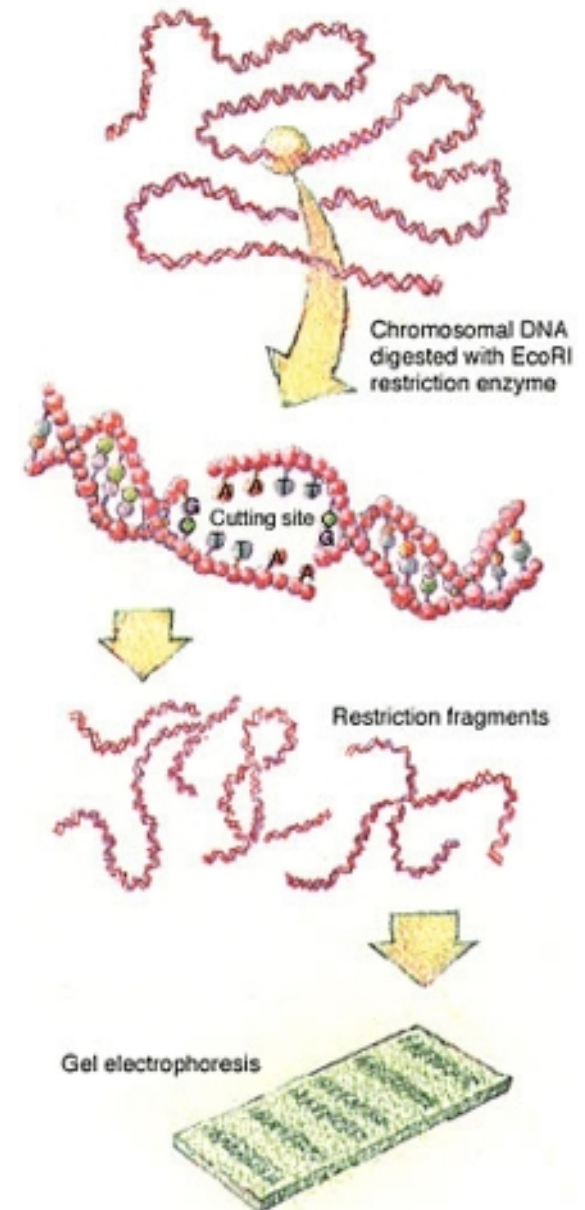
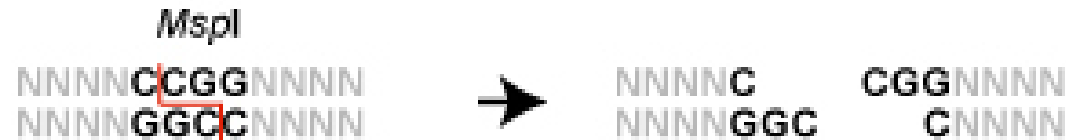


Blunt ends, 3' protruding ends, 5' protruding ends

Cut DNA with restriction enzymes

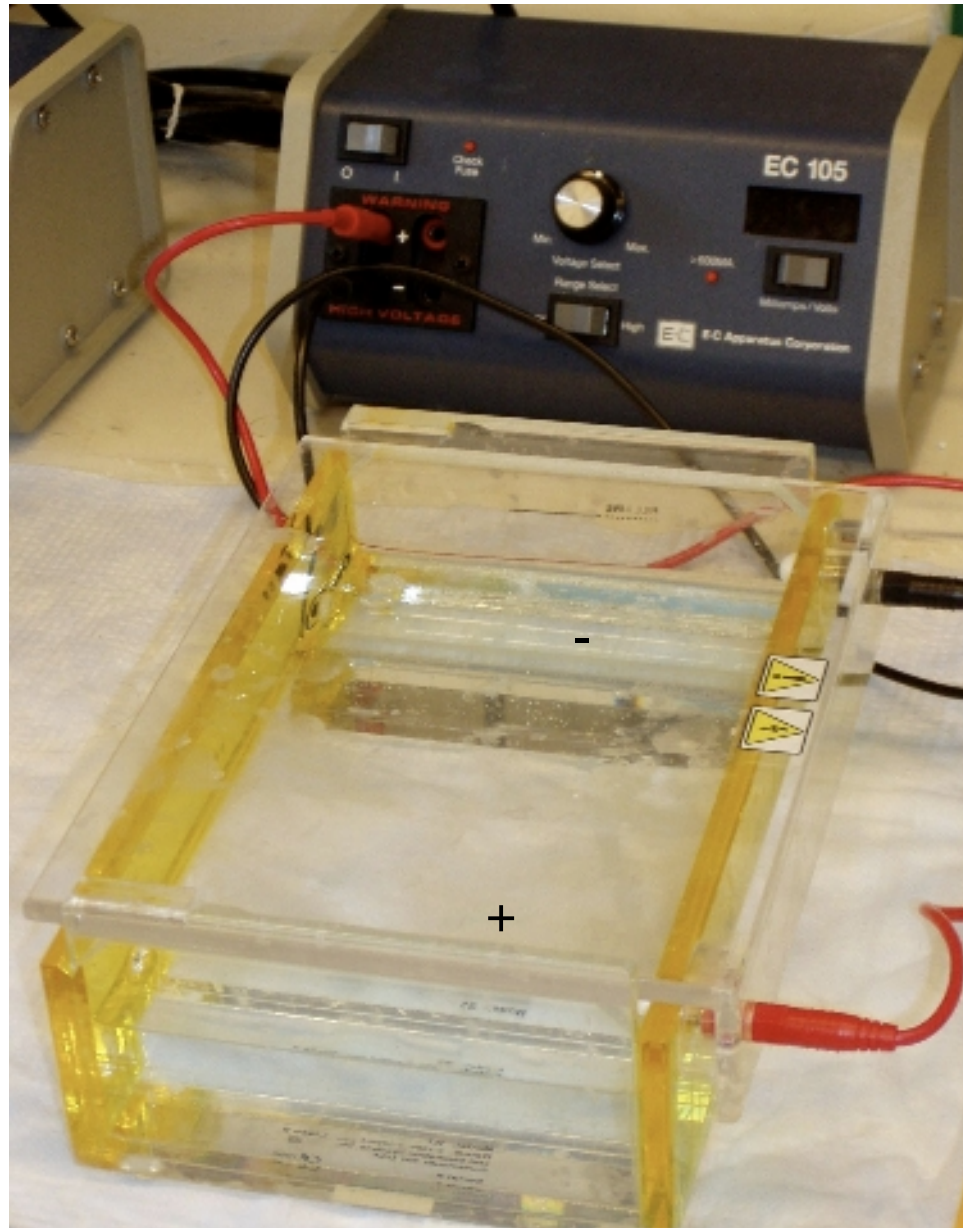
Sites de restriction

Résultats après coupure

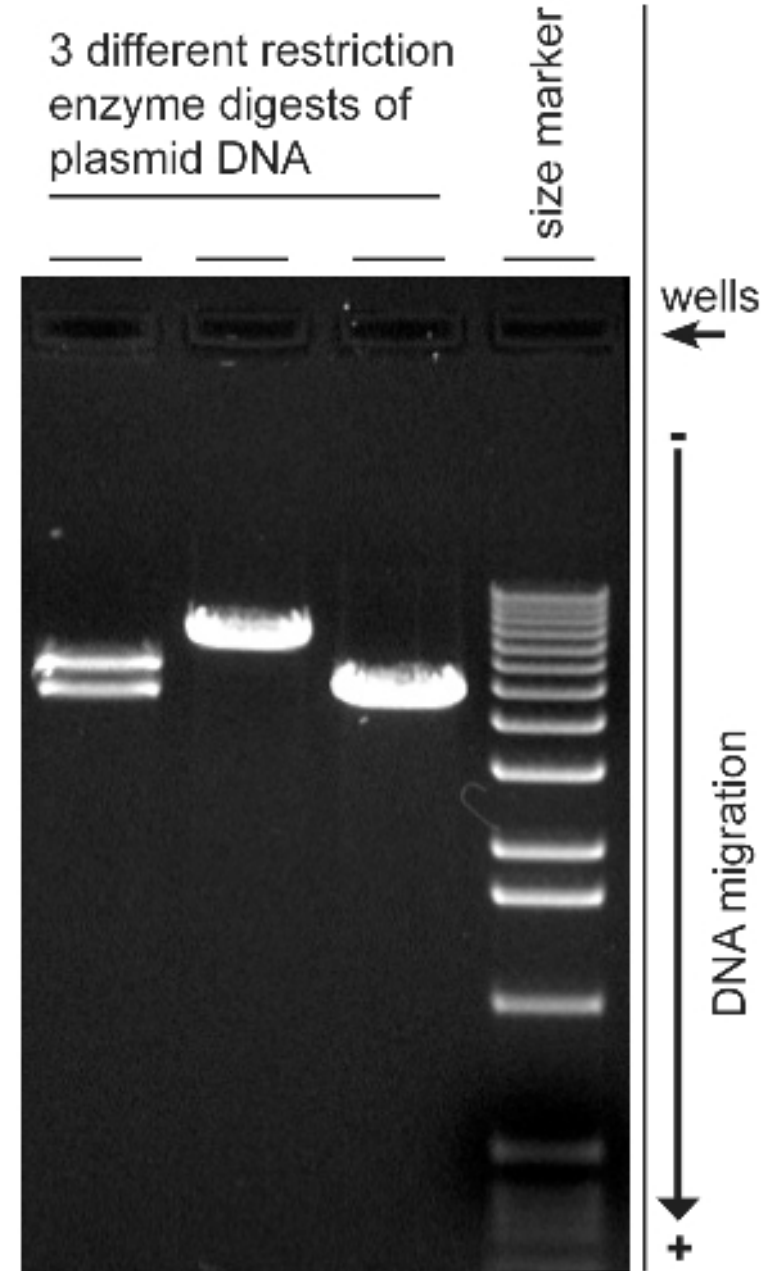


Blunt ends, 3' protruding ends, 5' protruding ends

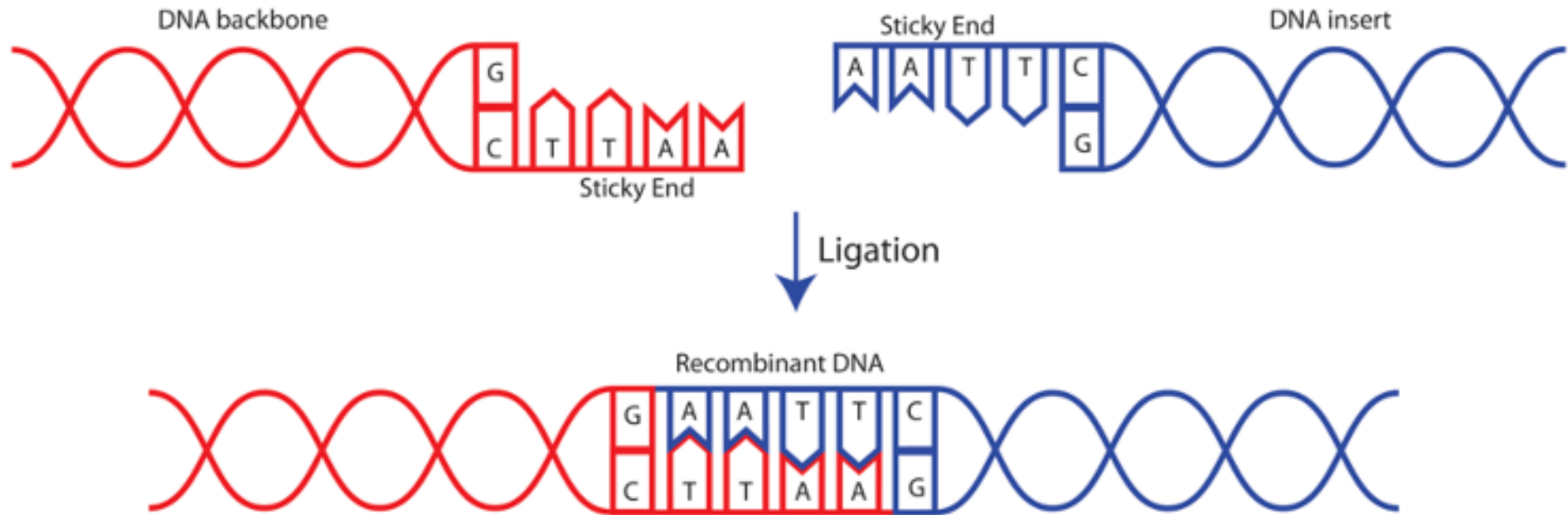
Examine length of DNA



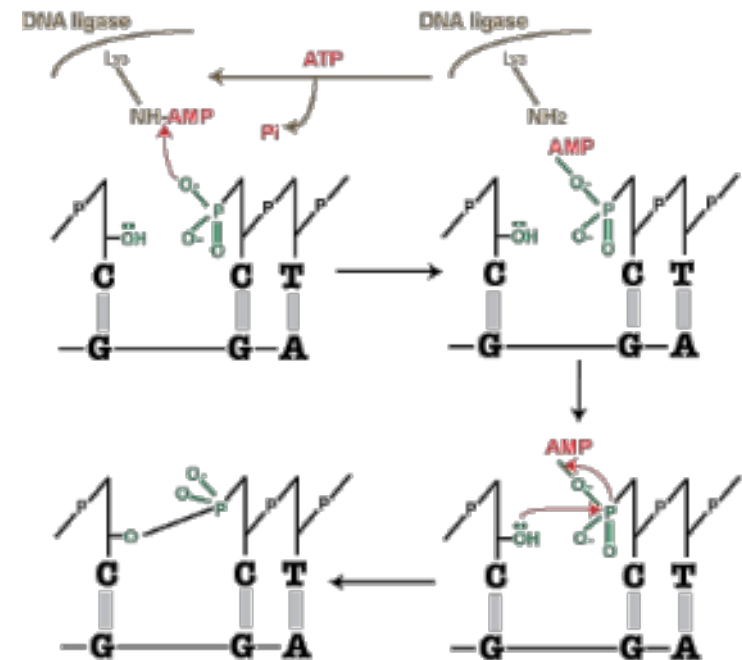
TAE (Tris-acetate-EDTA) buffer



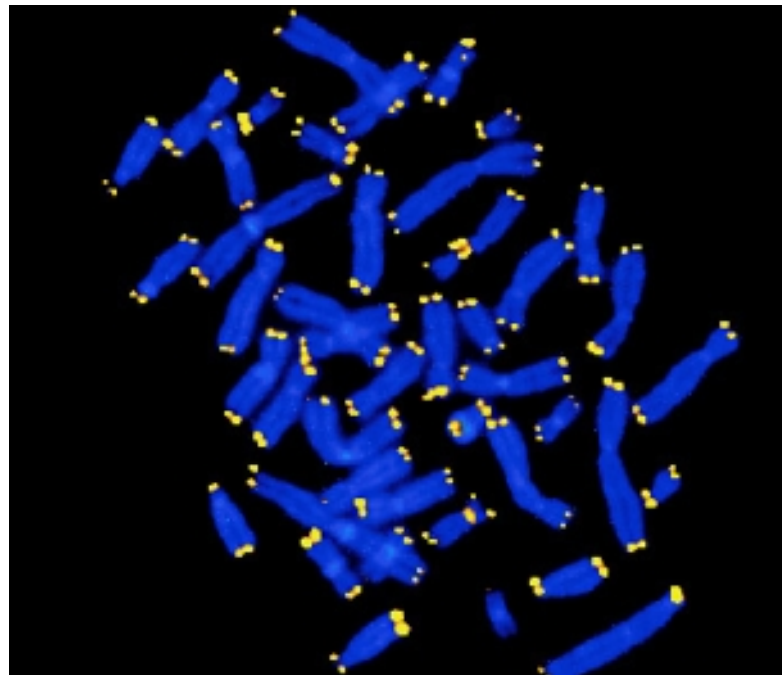
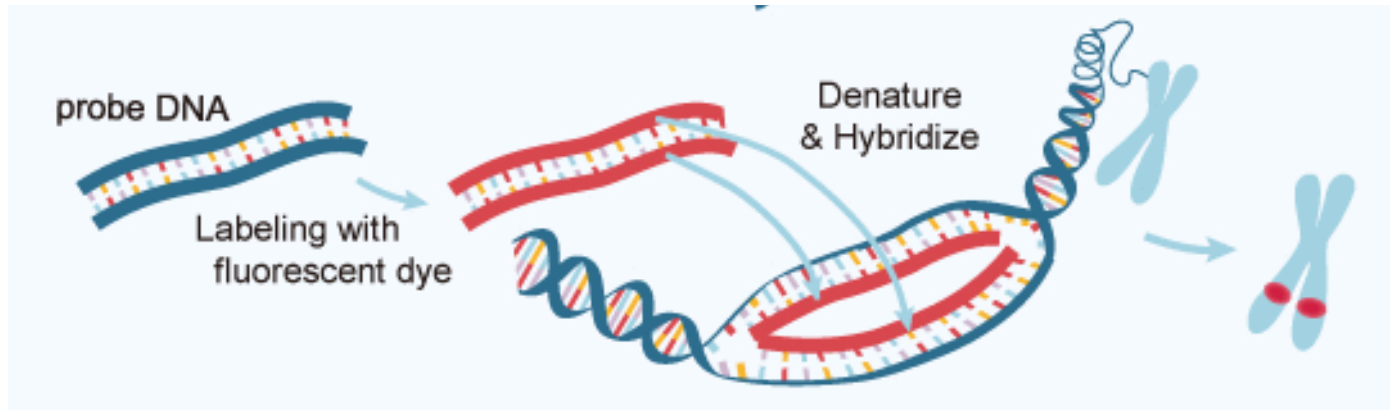
Ligate DNA



Fragments have to be phosphorylated but only on one strand
Dephosphorylate the vector to inhibit self-circularization



Probe DNA: Fluorescent In Situ Hybridization



Probes for telomere sequences

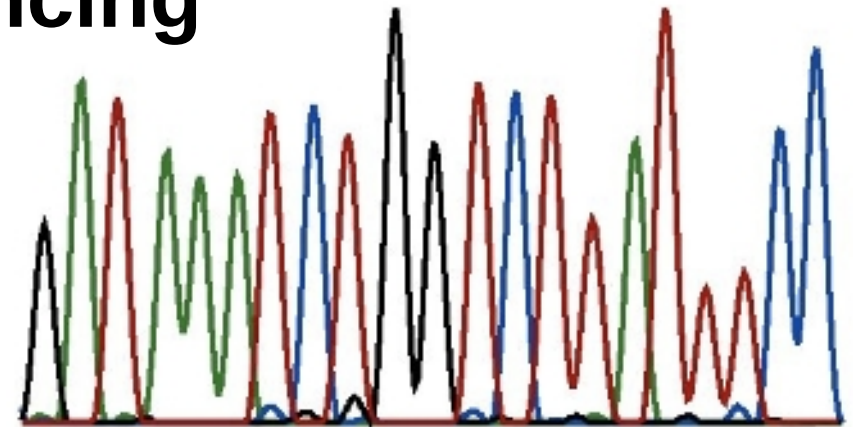
Sanger sequencing

800 bp long

Starts based on oligonucleotide (primer)

~4 euros per reaction

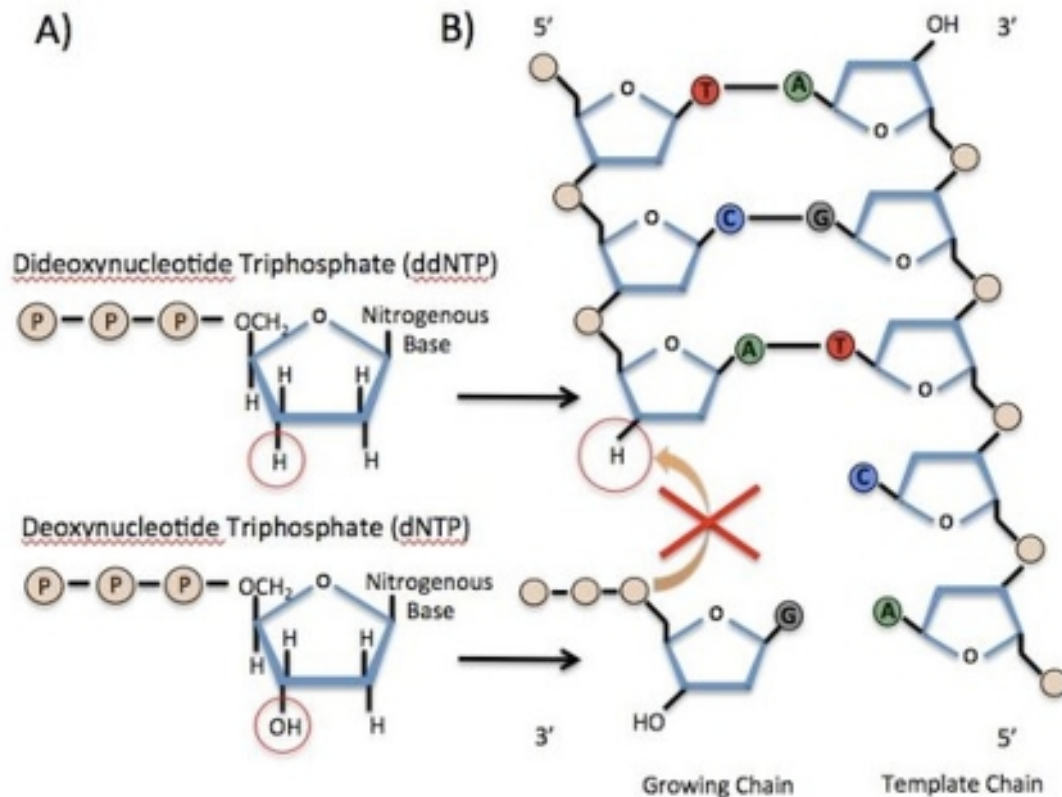
Dye terminator sequencing



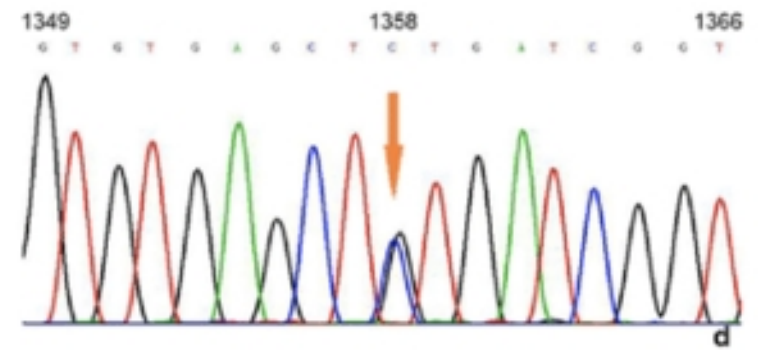
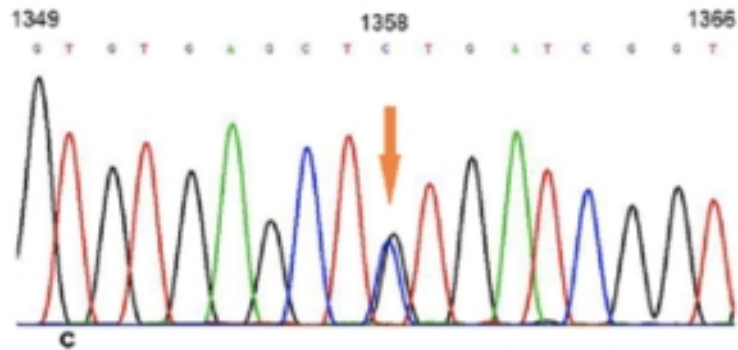
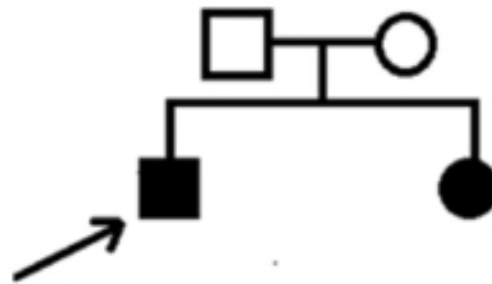
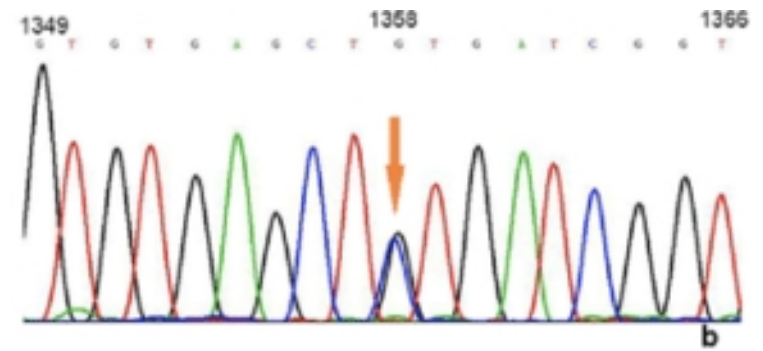
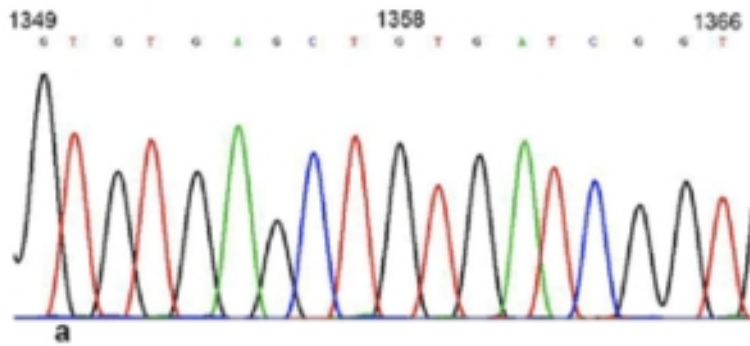
120 130
G A T A A A T C T G G T C T T A T T T C C

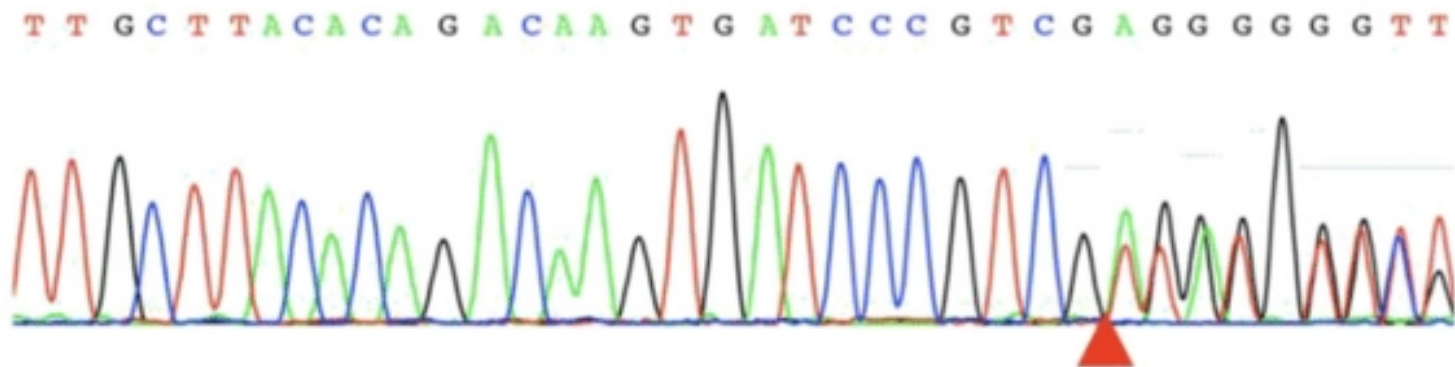
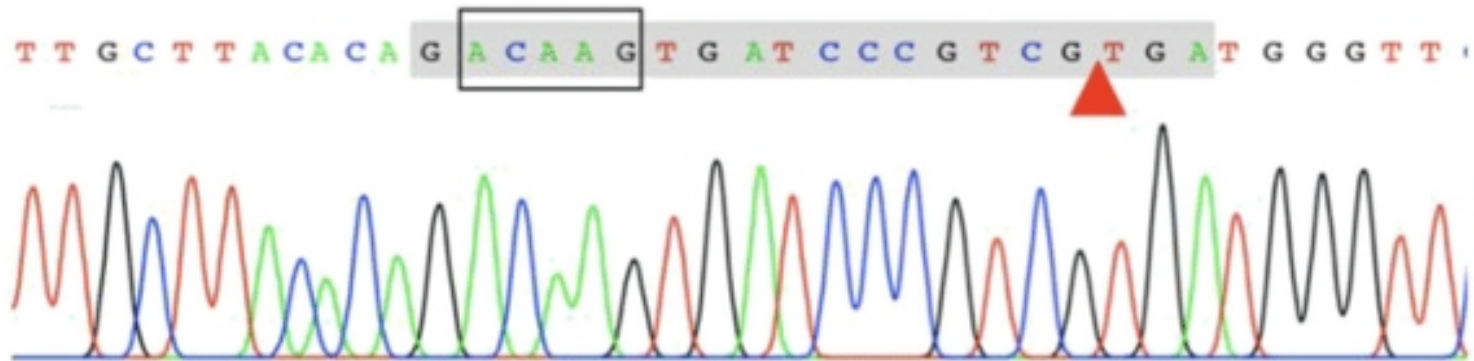
C) Template Sequence
3' GAGCAAATTCGATACATTATTGT... 5'
Primer
5' CTCGTTTAAG... 3'

CTCGTTTAAGG — G
CTCGTTTAAGGC — C
CTCGTTTAAGGGT — T
CTCGTTTAAGGGTA — A
CTCGTTTAAGGGTAT — T
CTCGTTTAAGGGTATG — G
CTCGTTTAAGGGTATGT — T
CTCGTTTAAGGGTATGTA — A
CTCGTTTAAGGGTATGTAA — A
CTCGTTTAAGGGTATGTAAT — T



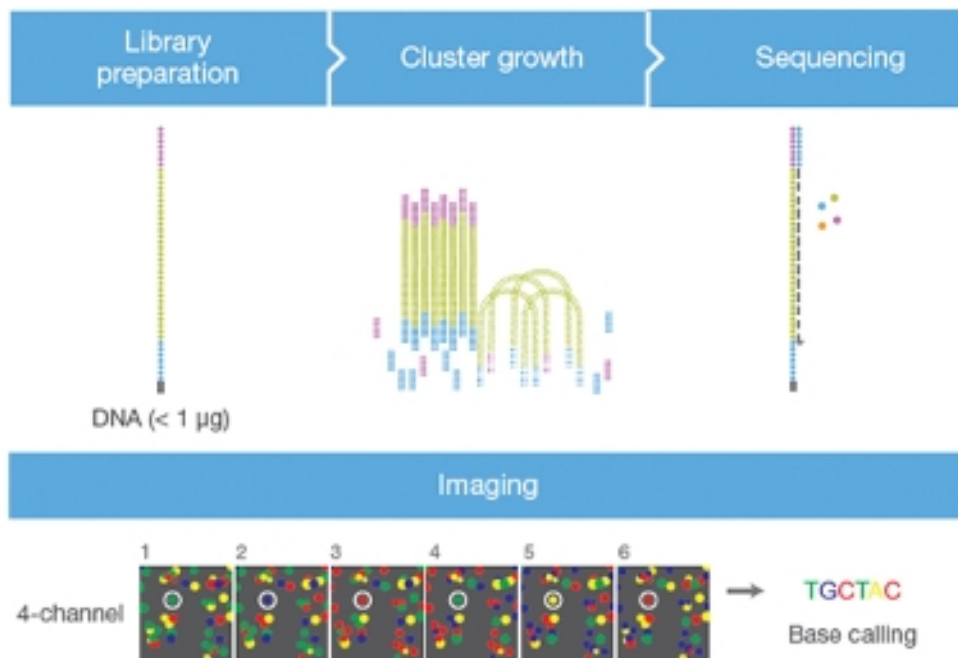
GTGTGAGCTGTGATCGGT





Illumina sequencing

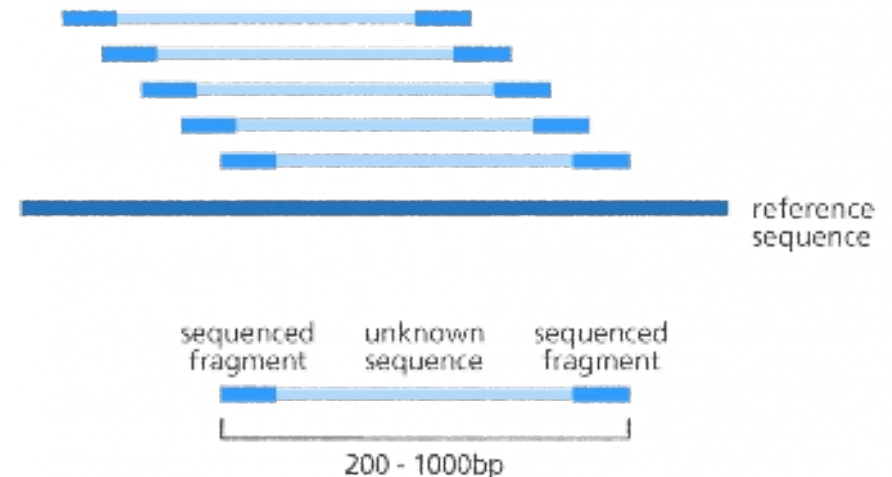
Millions of reads, each ~100 bp long
Starts at all possible positions
~500 euros per run



Single-end reads



Paired-end reads



For transcriptome: 2x 75 bp
For whole genome: 2x 150 bp

Manipulating RNA

**What can we do with
RNA ?**

What can we do with RNA ?

Extract, purify

Make more

RNA → DNA → RNA (reverse transcription, transcription)

Examine

Quantify

Examine length

Stain, probe

Sequence

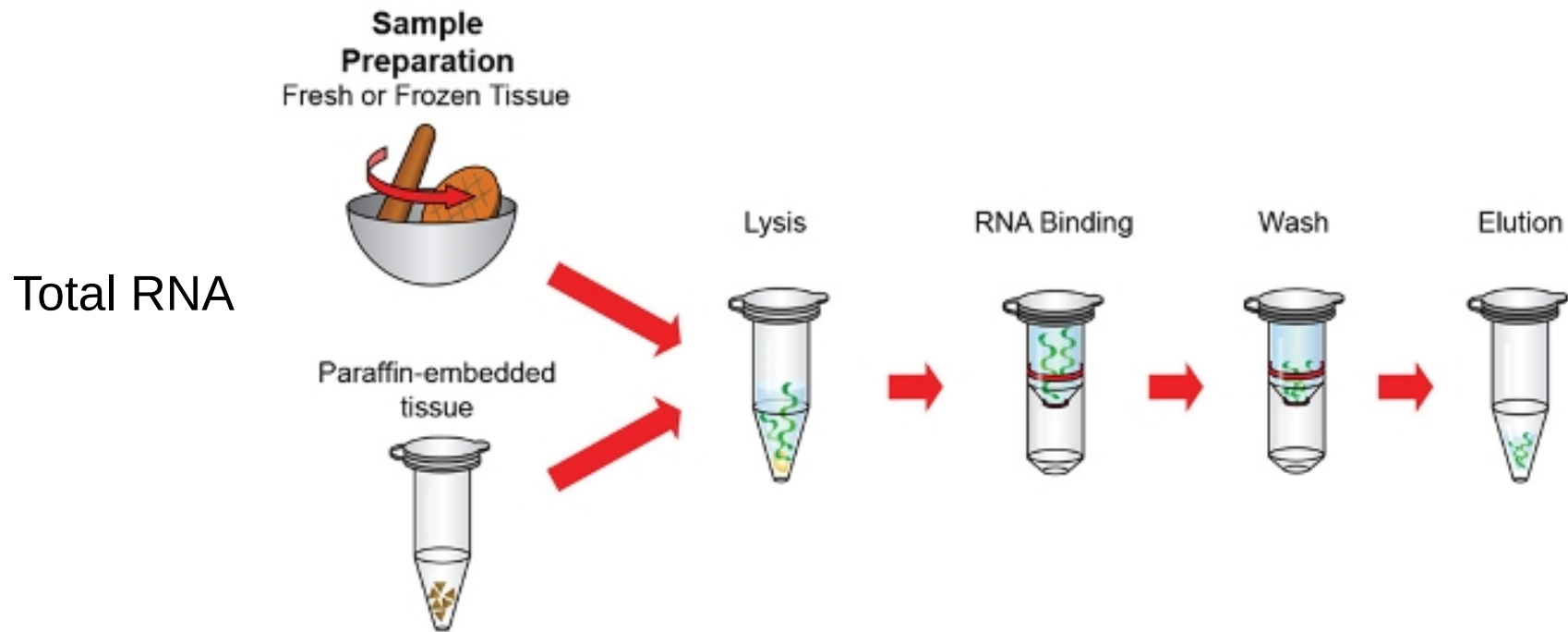
Examine 3D structure

Measure physical properties of RNA molecules

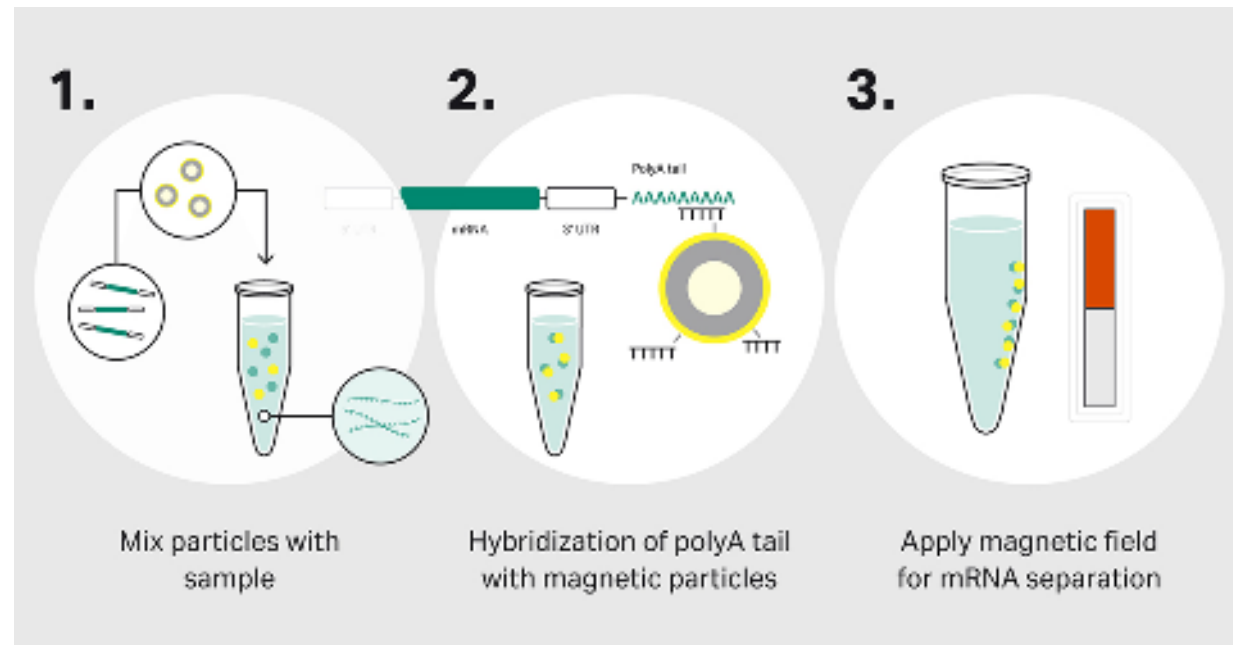
Modify

Mostly via DNA

Extract RNA

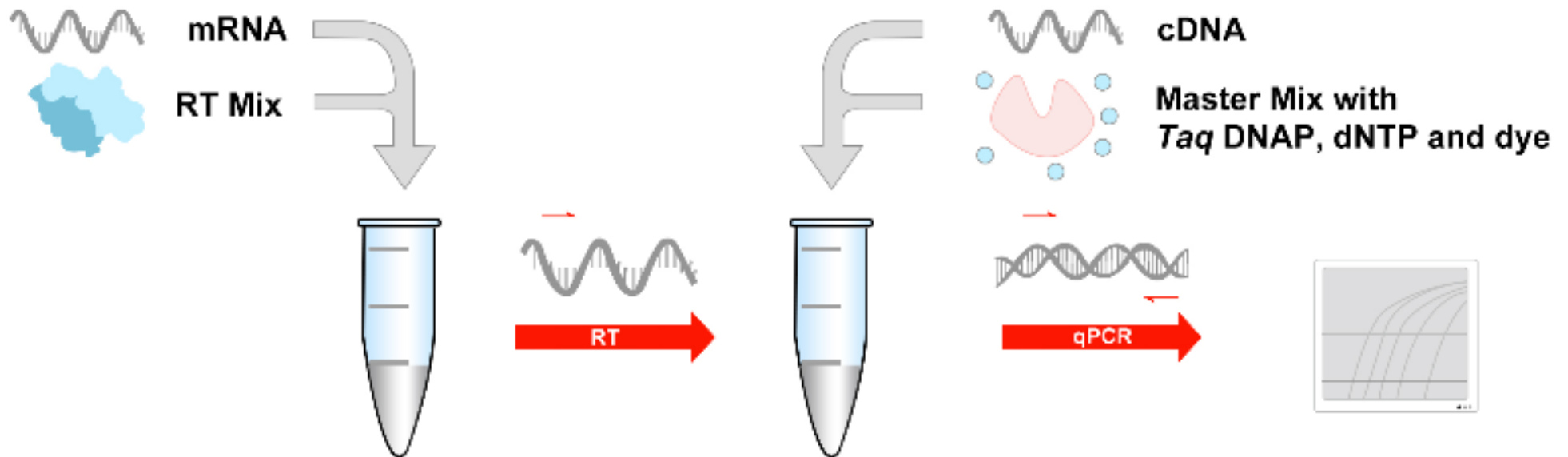


mRNA



RT-qPCR

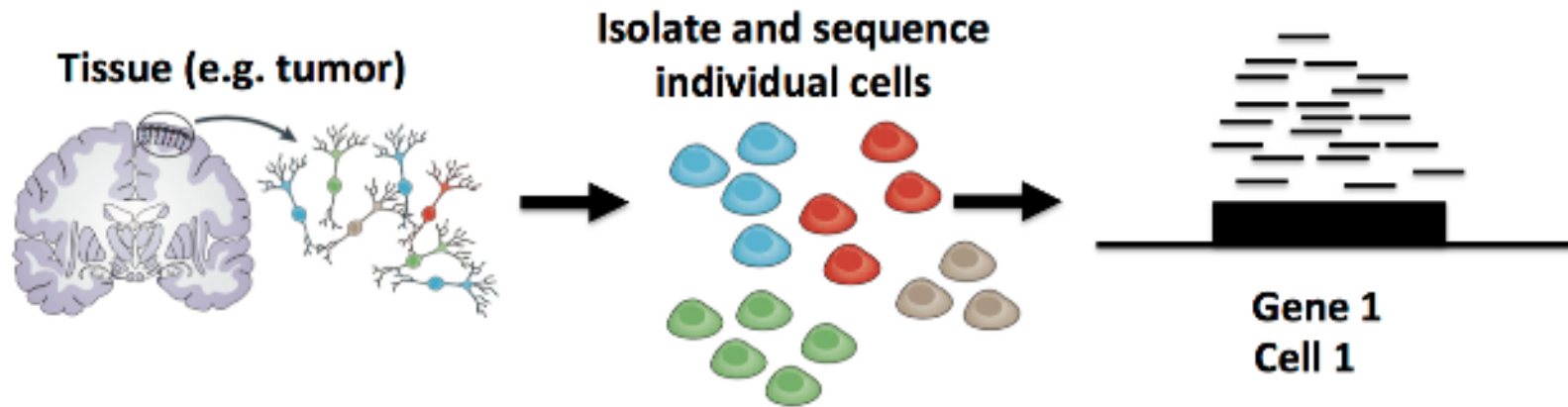
BlazeTaq™ Two-Step RT-qPCR Kit



RNAseq

Starting with tissues/organs/single cells

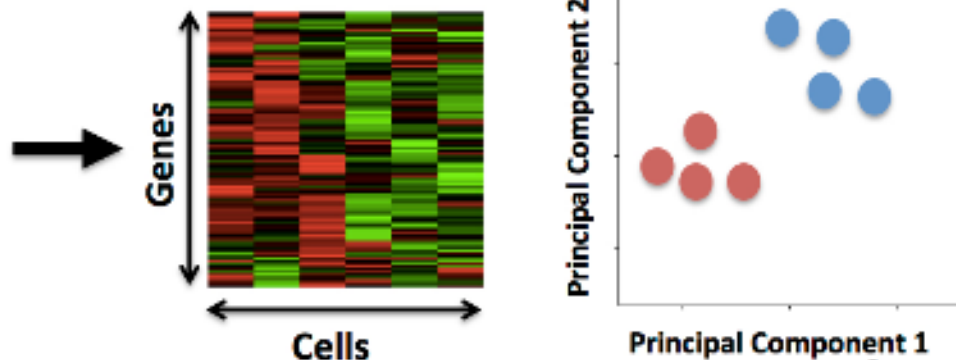
Single-cell RNA-Seq (scRNA-Seq)



Read Counts

	Cell 1	Cell 2	...
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
...			

Compare gene expression profiles of single cells



DNA and its observable effects

The genotype-phenotype map

The distinction between genotype and phenotype is the basis of genetics

“The view of natural inheritance as realized by an act of transmission, viz., the transmission of the parent's (or ancestor's) personal qualities to the progeny, is the most naive and oldest conception of heredity.”

“All "types" of organisms, distinguishable by direct inspection or only by finer methods of measuring or description, may be characterized as "**phenotypes**.”

“ A "**genotype**" is the sum of all the "genes" in a gamete or in a zygote.”



Johansen 1911

Phenotype = observable attributes
of an individual

Genotype = inheritable genetic material
= DNA or RNA

How do genotypes map onto phenotypes ?

Aberration Types

Substitution

Insertion (CNV)

Deletion

Indel

Inversion

Translocation

Complex change

(Epigenetic change)

Aneuploidy = anomalous number of chromosomes

Estimation of mutation rates

Mutation accumulation lines, sequencing family trio, across a phylogeny

Coding versus cis-regulatory

Coding

Cis-regulatory

Gene loss

Gene amplification

(Gene rearrangement)

Different kinds of phenotypes

Morphology

Color

Size and shape

Presence/

absence

Position

Physiology

Behavior



Aristote, Historia animalium, book I, 2, 300BC

Genotype & Phenotype

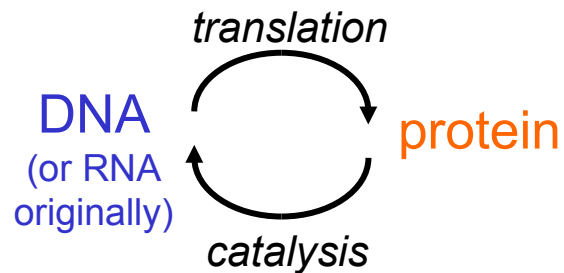
= what engenders = what is apparent

DNA/RNA

- Regulation of gene expression
- Biochemical reactions
- Subcellular architecture
- Assembly of cells
- Organism morphology and behavior

distinction appeared at the origin of life:

etc.



Francis Crick Central Dogma

A reductionist view of the GP relationship

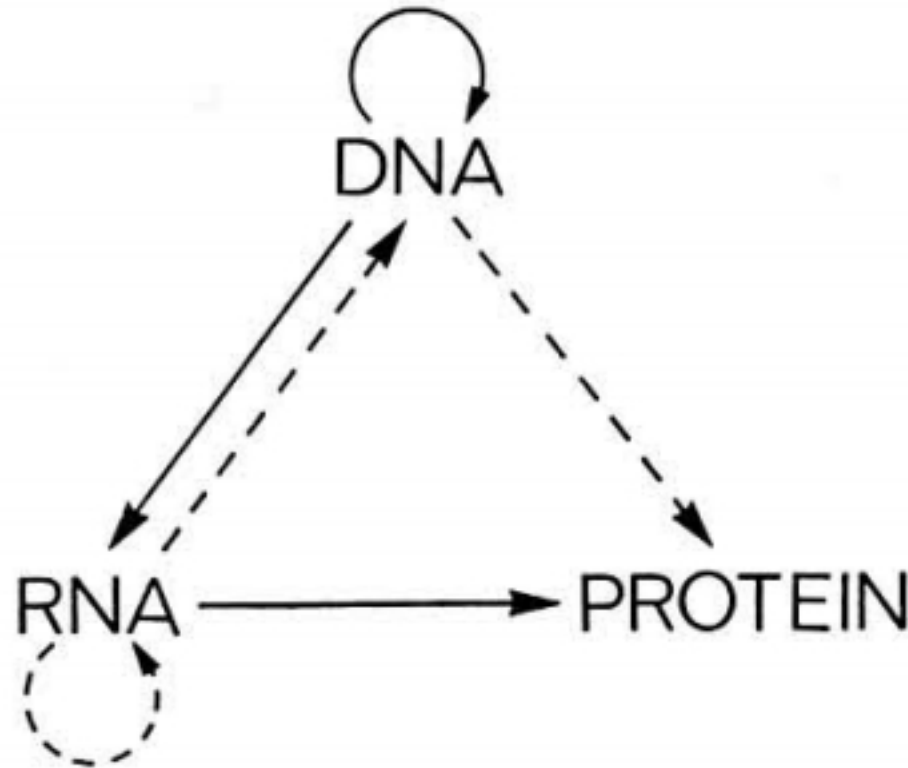


Fig. 3. A tentative classification for the present day. Solid arrows show general transfers; dotted arrows show special transfers. Again, the absent arrows are the undetected transfers specified by the central dogma.

How do genotypes map onto phenotypes ?

DEVELOPMENTAL BIOLOGY

EVOLUTIONARY GENETICS

Both are direct descendants of Morgan's school. Emphasis on genes.

How does an organism form from a single cell?

What makes one organism different from another one?

One of the central problems of biology is that of differentiation - how does an egg develop into a complex many-celled organism? That is, of course, the traditional major problem of embryology; but it also appears in genetics in the form of the question, “How do genes produce their effects?”

Sturtevant, 1932

How do genes produce observable traits?

Gene



Observable
character

Pax6 : an eye gene ?

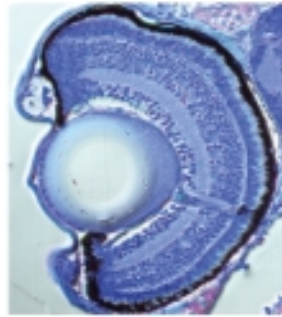
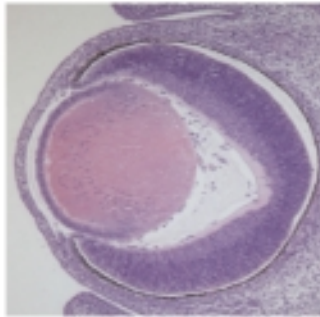
Human

Mouse

Zebrafish

Drosophila

WT

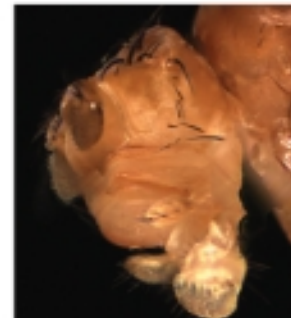
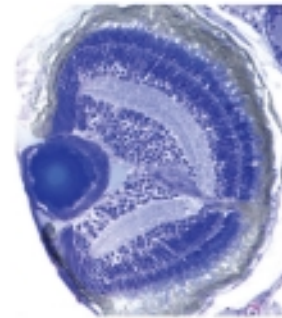
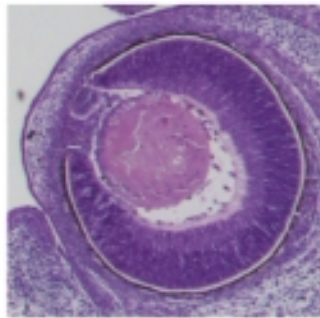
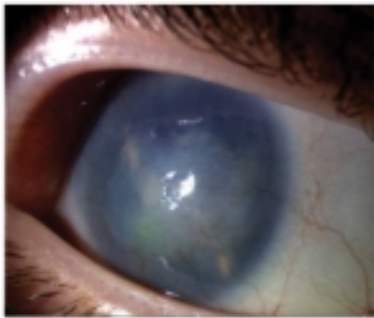


Drosophila



WT

mut



PAX6^{+/-}

Pax6^{-/-}

pax6b^{-/-}

ey^{-/-}

EQs

cornea opaque
iris absent
retina degenerate
lens opaque
aqueous humor of eyeball
increased pressure

eye decreased size
lens fused_to cornea
iris morphology
anterior chamber
absent

eye decreased size
lens decreased size
retina malformed

eye absent

**Ectopic expression
of Pax6**



Gene

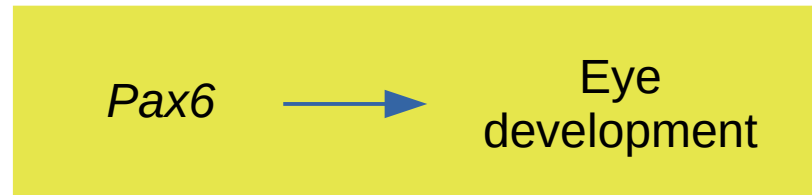


Observable
character

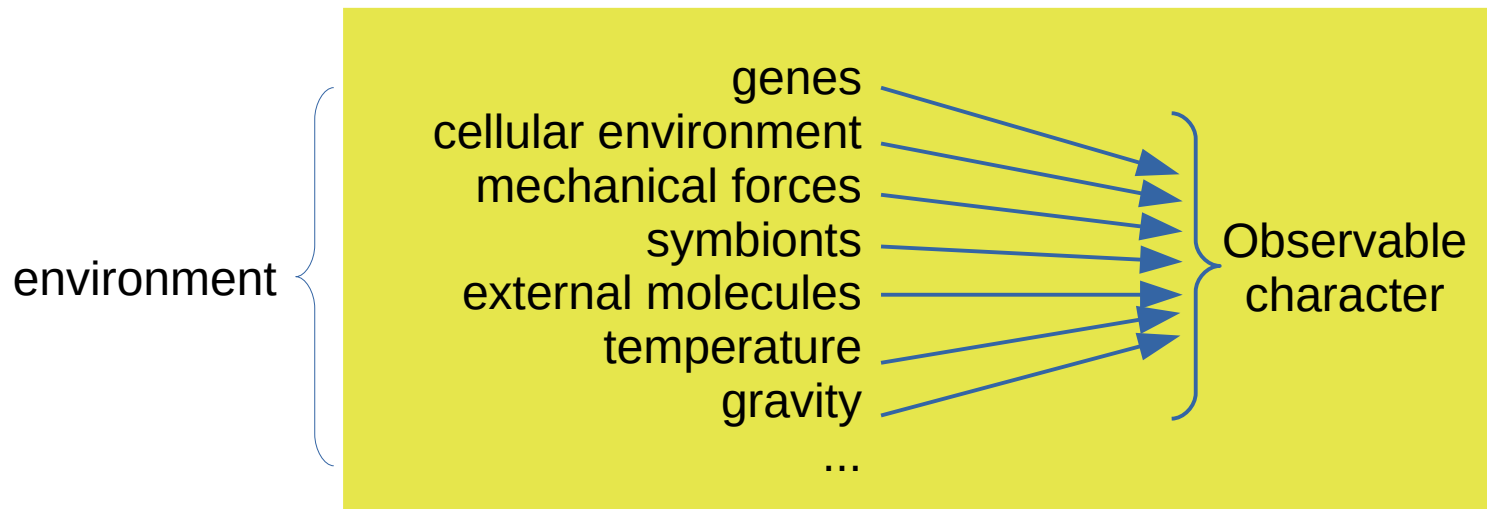
Pax6



Eye
development



Too simplistic



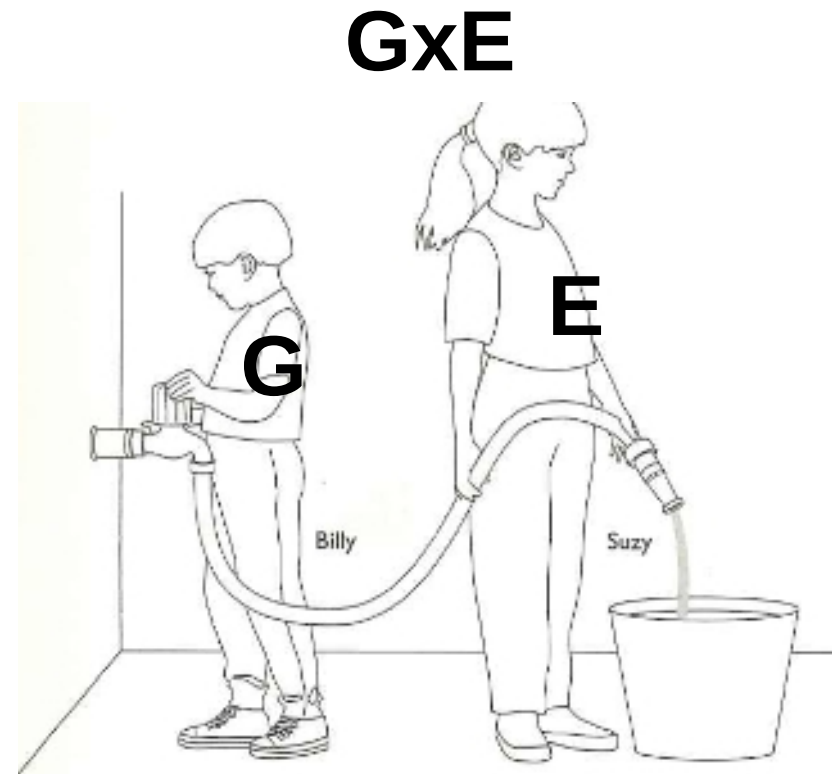
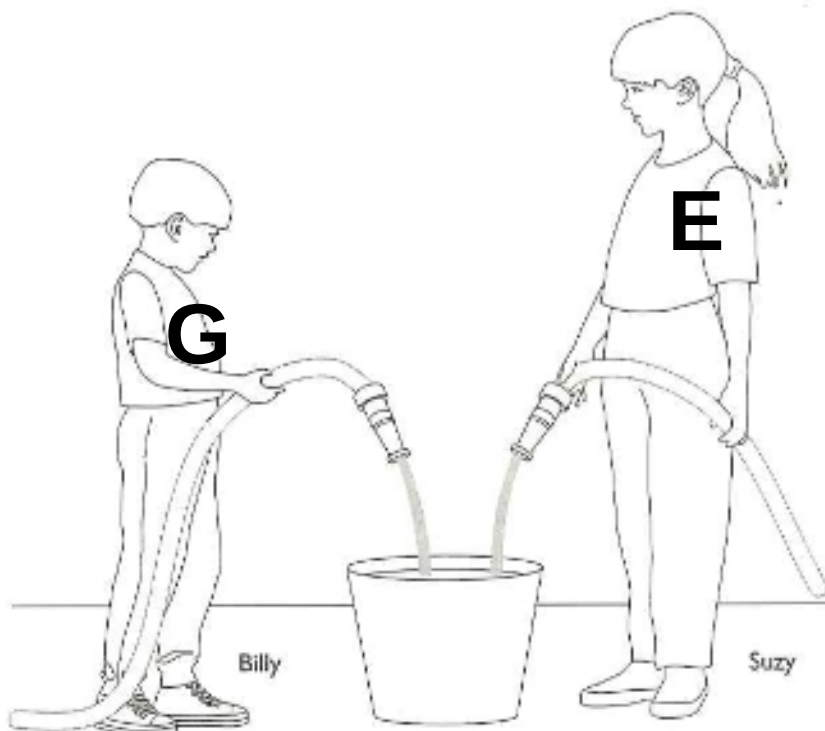
Better, but difficult to disentangle the effects

We all come from GxE (Genes x Environment)



Eero Mäntyranta, Finnish ski champion
7 Olympic medals in the 60s
Mutation enabling his blood to transport oxygen more efficiently
His family members were not Olympic champions

Impossible to quantify innate versus acquired influences

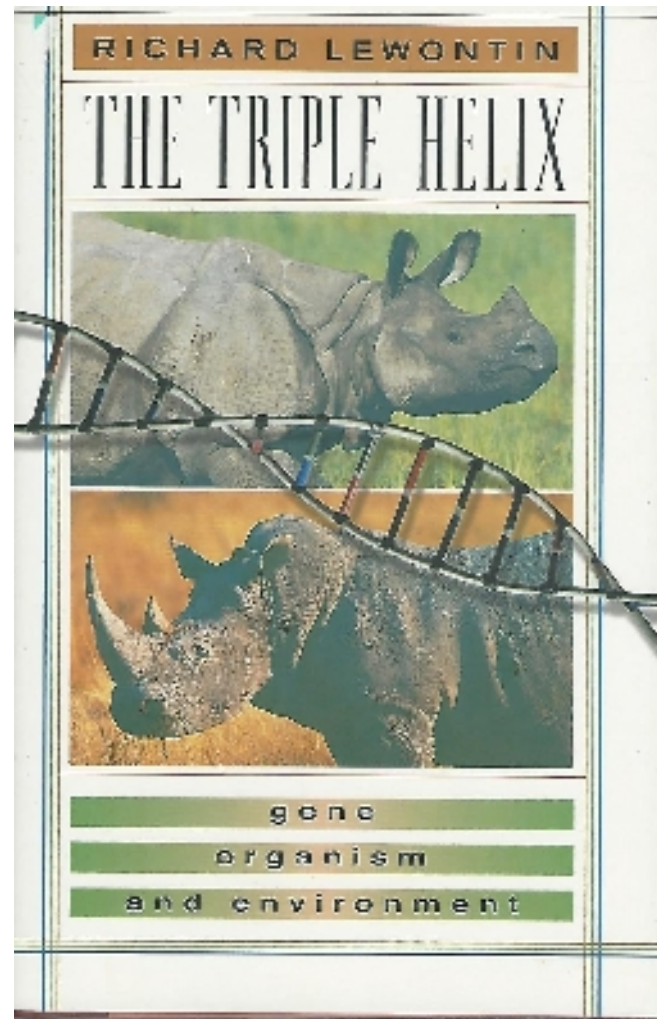


The zombie fly

Entomophthora muscae
Parasite of
Musca domestica



© D.R.



Exercices!